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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Bixa orellana* against Gonorrhea

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ABSTRACT

Phytochemicals are nothing but the non-nutritive compounds, obtained from plants. It has been reported that *Bixa orellana* plant extract is used to cure Gonorrhea. The plant extract contains different phytochemicals. Gonorrhea is caused by *Neisseria gonorrhoeae* bacteria. One of the key enzymes involved in its biochemical pathway is Glutathione transferase. The molecular docking of the phytochemicals with the enzyme was studied using biovia discovery studio. The strength of the interaction was evaluated based on –CDocker energy and –CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Phynol, Benzoic-acid, Phytol, Anthraquinone and Acitic Acid can effectively deactivate Glutathione transferase enzyme. Thereby interrupting the life cycle of *Neisseria gonorrhoeae*

Key Words: Phytochemical, biovia discovery studio, *Bixa orellana, Neisseria gonorrhoeae.*

INTRODUCTION

Today, there are wide changes occurred in life of all people. Malnutrition unhealthy diet, smoking, alcohol consuming, drug abuse stress etc, are the presentations of unhealthy life style. Besides that lives of citizens full with new challenges. Due to this kind of life style and bad food habits diseases like blood pressure, diabetes, obesity, skin disease etc occur.Nature has been a source of medicinal agents from the beginning and a very large number of modern drugs have been derived from natural sources (Veeresham C 2012). Natural product derived from plant as a source of drugs. Some of the chemical substances having some medicinal values of the plant that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plant based medicinal constituents can be derived from any part of the plant like bark, leaves,

flowers, roots, fruits and seeds. Various medicinal plants and their phytoextract have some anumerous medicinal properties like anti- oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc (Medini F, Bourgou S, Lalancette KG, Snoussi M, Mkadmini K, Cote I, abdelly C, Legault J and Ksouri R 2015). Phytochemical analysis, anti-oxidant, anti- inflammatory and anti-cancer activities of the halophyte *Limonium densiflorum* extracts on human cell lines and murine macrophages. Medicinal plants plays key role in human health care from the ancient time. About 80% of the world population relays on the use of traditional medicine many of the medicinal plant are used as spices and food item. They also play an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homeopathy, and aroma therapy.

Medicinal plants are the foundation of many drugs, Prescribed today on modern medicinal system about 25% of modern pharmaceutical drugs have botanical origin. The breast cancer fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona spp*, vincristrine and vinblastine from *Catharanthous roseus*, atropine from *Atropa belladonna* and codeine and morphine from *Papaver somniferum* (G. Schmeda-Hirschmann and A. R. De Arias 1992). Research need in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety biological activityand clinical efficacy of the numerous plants in common uses id required. Newly emerging scientific \ echniques and approaches have been used in the growing area of medicinal plant research for the investigation of constituents and determination of biological activity of medicinal plant.

Plant that demonstrated anti cancer, anti oxidant, anti-inflammatory immunostimulatory and anti microbial properties has received research attention (Sekhar M, Aneesh T, Varghese K, Vasudaven D and Revikumar K 2007). Herbalism A phenomenon of new age in medicine. *Bixa orellana* belongs to family Bixaceae. *Bixa orellana* seeds extract in used to cure disease like Gonorrhea (Raddatz-mota D, Perez-FloresLJ, CarrariF, Mendoza-Espinoza JA, Leon-SanchezDe Fd, Pinzon-Lopez LL, Godoy-Hernandez G and Rivera-Cabrera F 2017). Achiote (*Bixa orellana L.*): a natural source of pigment and vitamin E. *Bixa orellana* is known to contain phytochemicals like Phenol, Norbixin, Phytol, Acitic Acid, Benzoicacid, Anthraquinone, Farnesyl-acetone (Rather LJ and Mohammad F 2016). Phytochemistry, biological activities and potential of annatto in natural cocorant production for indutrial applications-A review. There is high possibility that these phytochemicals play a major role in curing Gonorrhea.

A group of bacteria belonging to genus *Neisseria* generaly caused Gonorrhea. Gram negative *Cocco bacillus* bacteria can causes the sexully trasmitted disease like Gonorrhea , in developing countries, if untreated, may causes infertility. Yellowish discharges from the penis with itching and burning indecent Gonorrhea in men. 50% or more women do not have any symptoms Gonorrhea causing symptoms,but in some cases burning, frequent urination, yellowish vaginal discharge, redness and swelling and itching of the vaginal area ane seen. It can lead to severepelvic infection which can causes sterility. Gonorrhea can causes eye infection, heart valves, arthritis etc complications in later life and if a infected mother give birth a baby than that baby has eye infection. It may also spread from skin-to-skin contact with an infected person. Different extracts of *Bixa orellana* plant are help to block the metabolic pathways of the microbe as a result growth of microbe is stop and it may leads to the death of that microbe (Abeck D and Johnson A. P 1987). Identification of surface-exposed proteins in *Neisseria gonorrhoeae.* This study focuses on the identification of the phytochemical of *Bixa orellana* responsible to cure Gonorrhea caused by *Neisseria gonorrhoeae.*

MATERIALS AND METHODS

Software Used

Discovery studio module of biovia software was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Plants produce phytochemicals as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, virus, fungi etc. These phytochemicals fight with the bacteria, virus etc which are threats to health when their plants or any parts were taken by humans. Some phytochemicals have been used as poisons and other as traditional medicine. Published work showed that plant *Bixa orillana* contain Phenol, Norbixin, Phytol, Acitic Acid, Benzoicacid, Anthraquinone, Farnesyl-acetone etc. It has already been established that *Bixa orellana* plant belongs to *Bixaceae* family has potential to help controlling sexually transmitted disease like Gonorrhea. This work is focused on identification of the particular responsible for inhibiting and controlling of Gonorrhea.

Enzyme Found in *Neisseria gonorrhoeae*

It has been reported that can cause as a result of *Neisseria* spp. Infestation. Various metabolic cycle have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database used to identify and list different enzymes found in *Neisseria spp.* Bacteria. It has been found that beta Glutathione transferase (protein data base code 4HOJ) is involved in Glutathione metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the microbial protein to successfully inhibit the microbe. The discovery studio module of the biovia software is using for identify molecular interaction and perform molecular docking. In this process, first the sdf files for the phytochemicals found in the *Bixa orellana* plant were downloaded from the website (www.molinstincts.com).The protein data base code of the Glutathione transferase enzyme was identified from the website (www.brenda-enzymes.org). The active site of the enzyme was identified via “receptor cavity” protocol found under “receptor -ligand interaction” menu. Molecular docking was done using the CDocker protocol of biovia software under “receptor-ligand interaction. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY”and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of the molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interaction with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Glutathione transferase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the non-bonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019 , 56(2), 111-121).

Table 2 shows that Glutathione transferase-Benzoic Acid interaction has the highest positive value of -CDOCKER energy (20.0048) and minimum value of the difference (2.4274) between - C DOCKER interaction energy and - C DOCKER energy followed by Phynol, Phytol, Anthraquinone and Acitic Acid. Thus the results indicated that Phynol,

Benzoic-Acid, Phytol, Anthraquinone and Acitic Acid can effectively deactivate the Glutathione transferase enzyme thereby interrupting the biological cycle of *Neisseria ssp.*. Higher positive values for Benzoic Acid indicated that it was the most active ingredient against *Neisseria ssp.*. On the other hand Farnesyl-acetone can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Norbixin cannot interact with Glutathione transferase enzyme. Thus, the key phytochemicals preventing Gonorrhea caused by *Neisseria sp.* are Phynol, Benzoic-acid, Phytol, Anthraquinone and Acitic Acid

CONCLUSION

It was previously known that *Bixa orellana* plant has medicinal action against Gonorrhea. Gonorrhea is caused by *Neisseria gonorrhoeae*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the Phytochemical (Phenol, Norbixin, Phytol, Acitic Acid, Benjoicacid, Anthraquinone, Farnesyl-acetone) which can have a significant interaction with the vital enzyme (Glutathione transferase) of the microbe. It was found that Phynol, Benzoic-acid, Phytol, Anthraquinone and Acitic acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Farnesyl-acetone were found to be not much effective in deactivating the enzyme of the microbe. Norbixin cannot deactivate the enzyme. Thus, this study could explain that the presence of Phynol, Benzoic-acid and Acitic acid provided the medicinal values to *Bixa orellana* against Gonorrhea caused by *Neisseria Sp.*.

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Table 1. Results of CDocking of Phytochemicals with Glutathione Transferase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Phenol	15.2179	17.4757	2.2578
2	Benzoic Acid	20.0048	22.4322	2.4274
3	Acitic Acid	13.5156	10.9485	2.5671
4	Phytol	10.8652	46.1363	35.2711
5	Anthraquinone	9.50948	19.477	9.93752
6	Farnesyl-acetone	-30.8607	41.025	71.8857
7	Norbixin	failed	Failed	NA

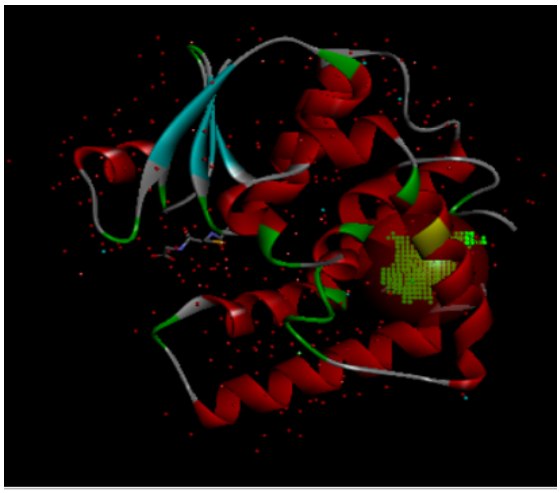

Figure 1. Active Site of Glutathione Transferase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Kaunch beej* against Thymidylate Synthase of *Bordetella Pertussis* Causing Cough

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ABSTRACT

Kaunch beej is an economically important plant belonging to family Fabaceae having both food and medicinal value. Phytochemicals are chemical compounds produced by plants. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been estabilished yet. It has been rumored that *Kaunch beej* plant extract is used to cure cough. The plant extract contains different phytochemicals like Ascorbic acid, Beta-carboline, Bufotenine, Galic acid, L-dopa, seratonine, s-nicotine, Linoleic acid, Palmitic acid, Stearic acid. Cough is caused by the microbe *Bordetella pertussis.* One of the key enzymes involved in its biochemical pathway is pyrimidine metabolism pathway. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals L-dopa can effectively deactivate the thymidylate synthase enzyme thereby interrupting the life cycle of *Bordetella pertussis.*

Key Words: phytochemical, Biovia, Discovery studio, *Bordetella pertussis, Kaunch beej*

INTRODUCTION

In ancient days, life of was natural, slow, difficult at times but healthy. Today, in modern day to day life, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition due to modern requirements, weak body due to less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little cautiousness, small changes in lifestyle and care for body if taken, we can prevent these lifestyle related diseases from increasing.
Nature and our environment has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants

lies in some chemical substances found in plant extract that produce a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011).

Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items from ancient days in countries like India, China, Africa etc. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Devi P.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Herbal medicine is the oldest form of medicinal treatment. People used medicinal plants for different therapeutic purposes from old days. Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus,* atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010).

Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Kaunch beej belongs to family Fabaceae and genus Mucun. [1]. *Kaunch beej* extract is used to cure disease like cough (reference). *Kaunch beej* is known to contain phytochemicals like gallic acid, ascporbic acid, L-dopa, beta crboline, bufotenine, linoleic acid, s-nicotine, palmitic acid, serotonine, oleic acid, stearic acid etc. [3,4]. There is high possibility that these phytochemicals play a major role in curing cough. However, there is no report identifying the specific phytochemical responsible to cure cough. A group of bacteria belonging to genus Bordetella generally cause cough. They are circular shaped Gram negative and aerobic *CoccoabaciIli* bacteria. They are motile and express flagellum-like structure. *Bordetella* infection is an acute respiratory infection marked by severe, spasmodic coughing episodes during the paroxysmal phase. *Bordetella pertussis* bacteria typically live in animal and human lungs, throats and the upper part of the respiratory system. Humans become infected most frequently through contaminated water or food. [1, 2, 3]. This study focuses on the identification of the phytochemicals of *Kaunch beej* responsible to cure cough caused by *Bordetella pertussis* bacteria.

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Kaunch beej* contains phytochemicals like Ascorbic acid, Beta-carboline Bufotenine, Galic acid, L-dopa, seratonine, s-nicotine, Linoleic acid, Palmitic acid, Stearic acid etc. [3, 4]. It has already been established that *Kaunch beej* plant belonging to Fabaceae family has potential to help controlling cough. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Cough.

Enzyme found in *Bordetella pertussis*

It has been reported that cough can caused as a result of *Bordetella* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Bordetella pertussis* bacteria. It has been found that thymidylate synthase enzyme protein database code 1CI7, is involved in pyrimidine metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Kaunch beej* plant were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the thymidylate synthase enzyme was identified from the website (https:www.rcsb.org/structure/1CI7). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the thymidylate syntase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).

Table 1 shows that glycerol thymidylate synthase- L-dopa interaction has the highest positive value of -CDOCKER energy (32.5141) and minimum value of the difference (18.571) between - C DOCKER interaction energy and - C DOCKER energy.Thus the results indicated that L-dopa can effectively deactivate the Thymidylate synthase enzyme thereby interrupting the biological cycle of *Bodetella pertussis*. Higher positive values for L-dopa indicated that it was the most active ingredient against *Bodetella pertussis*. On the other hand, Galic acid can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Lnoleic acid, palmitic acid, stearic acid cannot interact with thymidylate synthase enzyme. Thus, the key phytochemical preventing diarrhea caused by *Bodetella pertussis* is L-dopa.

CONCLUSION

Kaunch beej is one of the most important plants with highly potent pharmacological activities. It was previously known that *Kaunch beej* plant has medicinal action against cough. Cough is caused by *Bordrtella prtussis*. This plant has some specific phytochemicals which are responsible for curing cough. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical Ascorbic acid, Beta-carboline, Bufotenine, Galic acid, L-dopa, seratonine, s-nicotine, Linoleic acid, Palmitic acid, Stearic acid, which can have a significant interaction with the vital enzyme thymidylate synthase of the microbe. It was found that L-dopa can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Ascporbic acid, beta carboline, bufotenine, seratonine, s-nicotine cannot deactivate the enzyme. Thus, this study could explain that the presence of L-dopa provided the medicinal values to *Kaunch beej* against diarrhea caused by *Bordetella pertussis*.

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Table 1. Results of CDocking of phytochemicals with thymidylate synthase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Ascorbic acid	-58.1466	-11.2769	46.87
2	Beta-carboline	-233.623	-129.922	103.7
3	Bufotenine	-252.901	-134.417	118.492
4	Galic acid	-35.8348	18.1646	53.99
5	L-dopa	32.5141	51.0821	18.571
6	seratonine	-123.605	-51.3676	72.24
7	s-nicotine	-380.096	-108.329	271.77

8	Linoleic acid	Failed	Failed	NA
9	Palmitic acid	Failed	Failed	NA
10	Stearic acid	Failed	Failed	NA

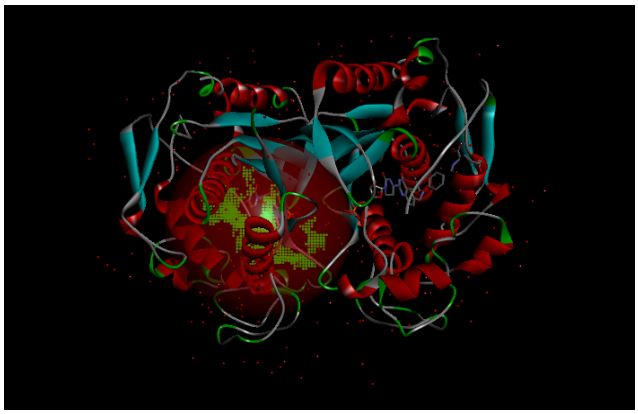

Figure 1. Active site thymidylate synthase enzyme

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Tinospora cordifolia* against Shikimate Dehydrogenase of *Escherichia coli* against Diarrhea

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ABSTRACT

Tinospora cordifolia is an economically important plant belonging to family menispermaceae having both food and medicinal value. Phytochemicals are chemical compounds produced by plants. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been estabilished yet. Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Tinospora cordifolia* plant extract is used to cure diarrhea. The plant extract contains different phytochemicals like Berberine, Choline, Columbin, Kaempferol, Palmatine, Phenol, quercetin, Syringing, Heptacosanol, Nanocosan, Sitosterol. Diarrhea is caused by *Escherichia coli.* One of the key enzymes involved in its biochemical pathway is Phenylalanine, tyrosine and tryptophan biosynthesis pathway. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals quercetin can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *E. coli.*

Key Words: phytochemical, Biovia, Discovery studio, *Tinospora cordifolia, E.coli*

INTRODUCTION

Life in olden days was natural, slow, difficult at times but healthy. Today, in day to day life, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition for modern standard, weak body due to less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little cautiousness, small changes in lifestyle and care for health if taken, we can prevent these lifestyle related diseases from increasing.

Nature and environment has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014).

Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Devi P.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus,* atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Tinospora cordifolia belongs to family menispermaceae. *Tinospora cordifolia* leaves extract is used to cure disease like diarrhoea (reference). *Tinospora cordifolia* is known to contain phytochemicals like Berberine, Choline, Columbin, Kaempferol, Palmatine, Phenol, quercetin, Syringing, Heptacosanol, Nanocosan, Sitosterol etc. (reference). There is high possibility that these phytochemicals play a major role in curing diarrhoea. However, there is no report identifying the specific phytochemical responsible to cure diarrhoea. A group of bacteria belonging to genus *Escherichia* generally cause diarrhoea. [6]. They are rod shaped Gram negative bacteria. *E.coli* infection is a common bacterial disease that affects the intestinal tract. *E.coli* bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food. [6]. This study focuses on the identification of the phytochemical of *Tinospora cordifoilia* responsible to cure diarrhea caused by *E. coli.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Tinospora cordifoilia* contains Berberine, Choline, Columbin, Kaempferol, Palmatine, Phenol, quercetin, Syringing, Heptacosanol, Nanocosan, Sitosterol .[4,5]. It has already been established that *Tinospora cordifoilia* plant belonging to Menispermaceae family has potential to help controlling diarrhea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of diarrhea. [1, 2, 3].

Enzyme Found in *E.coli*

It has been reported that diarrhea can caused as a result of *E.coli* infecion. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *E. coli* bacteria. It has been found that shikimate dehydrogenase enzyme protein database code 1NYT, is involved in Phenylalanine, tyrosine and tryptophan biosynthesis metabolism pathway (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Tinospora cordifolia* plant were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the Shikimate dehydrogenase enzyme was identified from the website (https:www.rcsb.org/structure/1NYT). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the shikimate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019 , 56(2), 111-121).

Table 1 shows that shikimate dehydrogenase-quercetin interaction has the highest positive value of -CDOCKER energy (23.9742) and minimum value of the difference (10.6) between - C DOCKER interaction energy and - C DOCKER energy followed by choline, kaempferol, phenol respectively. Thus the results indicated that quercetin,

choline, kaempferol and phenol can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *E.coli.* Higher positive values for quercetin indicated that it was the most active ingredient against *E.coli.* On the other hand berberine, columbin, magnoflovine can deactivate the enzyme to a small extent (negative-CDocker energy but positive -CDocker interaction energy). Syringing, Heptacosanol, Nanocosan, Sitosterol cannot interact with shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing diarrhea caused by *E.coli* are quercetin, choline, kaempferol and phenol.

CONCLUSION

Tinospora cordifoilia is one of the most important plants with highly potent pharmacological activities. It was previously known that *Tinospora cordifoilia* plant has medicinal action against diarrhea. Diarrhea is caused by *E.coli.* This plant has some specific phytochemicals which are responsible for curing diarrhea. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Berberine, Choline, Columbin, Kaempferol, Palmatine, Phenol, quercetin, Syringing, Heptacosanol, Nanocosan, Sitosterol) which can have a significant interaction with the vital enzyme shikimate dehydrogenase of the microbe. It was found that quercetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Palmatin and syingin were found to be not much effective in deactivating the enzyme of the microbe. Heptacosanol, Nanocosan, Sitosterol cannot deactivate the enzyme. Thus, this study could explain that the presence of quercetin provided the medicinal values to *Tinospora cordifoilia* against diarrhea caused by *E.coli.*

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Table 1. Results of CDocking of phytochemicals with Shikimate dehydrogenase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Berberine	-28.585	33.1138	61.69
2	Choline	22.4027	33.5952	11.19
3	Columbin	-81.3747	14.8227	96.19
4	Kaempferol	19.2729	27.5639	8.29
5	Magnoflorine	-6.6454	33.7726	40.41
6	Palmatine	-23.0642	-31.5551	8.49
7	Phenol	14.797	17.0368	2.24

8	Quercetin	23.9742	34.5755	10.6
9	Syringing	-578.765	-60.8854	517.58
10	Heptacosanol	Failed	Failed	NA
11	Nanocosan	Failed	Failed	NA
12	Sitosterol	Failed	Failed	NA

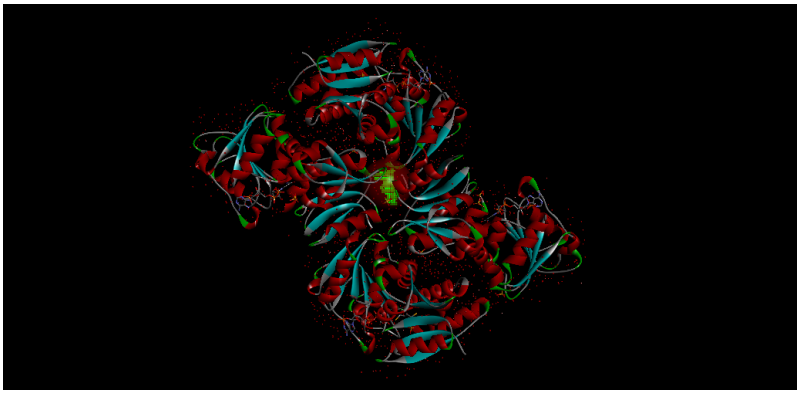

Figure 1. Active site of Shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Alpinia galanga* against Diarrhoea

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ABSTRACT

Phytochemicals are Secondary metabolites obtained from plants through various metabolic pathway. It has been reported that *Alpinia galanga* plant extract is used to cure diarrhoea. The plant extract contains different phytochemicals. Diarrhoea is caused by *Escherichia coli.* One of the key enzymes involved in its biochemical pathway is Shikimate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. Negative values for —CDocker energy and positive values for —CDocker interaction energy for all the phytochemicals of *Alpinia galanga* indicated that these phytochemicals are least effective in deactivating the Shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Escherichia coli*.

Key Words: Phytochemical, Biovia, Discovery studio, *Alpinia galanga, Escherichia coli.*

INTRODUCTION

The faster and developed life styles affects the health of an individual. Now a days lifestyles are an important factor for health. According to WHO 60% health factor is directly related with way of living (Ziglio E et al.,2004). Millions of people follow unhealthy lifestyle like unhealthy diet, smoking, alcohol consuming, drug abuse, stress and so on. Hence, they are encountered with illness, disability and problems like joint pain, cardiovascular disease, hypertension, overweight, skin lesions, low vision and low hearing problems in early age. A little change in way of living can prevent these diseases from annexing our society (Farhud DD, 2001). From Vedic period nature is the best source of medicine. According to the great scholar Charaka, every plant has its own medicinal properties. The Charaka samhita provides the information about diagnosis and treatment of diseases by using plant (Mazars G et al.,

2006). Medicinal plants are the major constituents of indigenous natural medicine, modern medicine, food supplements, bioactive principles, pharmaceutical intermediates and synthetic drugs (Neto et al.,2002) . Medicinal plants are used for therapeutic purposes to cure diseases. Over 90% of traditional medicine recipes or remedies contain medicinal plants.

Various medicinal plants and their non nutritive chemicals have shown numerous medicinal properties like anti-oxidant, anti inflammatory, anti-cancer, anti-microbial, antidiabetic action etc (Wiart C, 2006). It is estimated that up to four billion people living in the world use herbal medicinal products as a primary source of health care and traditional medical practice which involves the use of herbs which is viewed as an integral part of the culture in those communities (Bandaranayake, 2006).

Phytochemicals are compound that are produced by plants, they are biologically active but not nutritive. Phytochemicals act as natural defence mechanism in their host cell. It is also called as phytonutrients and secondary metabolites. Plant based chemical constituent can be derived from any part of plant like bark, leaves, flower, roots, fruits and seeds (D B Jack, 1997). *Alpinia galanga* belongs to family zingiberaceae (Saeio k et al., 2011). Galangal root extract is used to cure disease like Diarrhoea. *Alpinia galanga* is known to contain phytochemicals like β-farnesene, α-fenchyl acetate, β-bisabolene, β-bergamotene, β-pinene, 1-acetoxychavicol acetate etc (Chouni A et al., 2018). The rhizome of galangal plant is used in china for relieving stomach ache, treating colds and to reduce swelling. The bacteria *Escherichia coli* generally cause diarrhoea. It is a Gram negative, rod-shaped, facultative anaerobic bacterium (Nataro JP et al., 1998). Diarrhoea is mainly transmitted through contaminated food or water. This study focuses on the identification of the phytochemical of *Alpinia galanga responsible* to cure diarrhoea caused by *Escherichia coli.*

MATERIALS AND METHODS

Soft wares used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction. Biovia is the scientific brand of Dassault Systemes that is focused on the Virtual biosphere and Materials. Biovia is commited to enhancing and speeding innovation, improving productivity. Biovia is used for reducing cost and accelerating product development.The Discovery Studio module of Biovia is used for identifying molecular interaction and perform molecular docking of phytochemicals from plant extract that act as a ligand and form strong covalent bond with bacterial protein to successfully inhibit microbe.

List of Phytochemicals

Plants secret phytochemicals as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc., When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Galangal contains β-farnesene, α-fenchyl acetate, β-bisabolene, β-bergamotene, β-pinene, etc. It has already been established that Galangal plant belonging to Zingiberaceae family has potential to help controlling diarrhoea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of diarrhoea.

Enzyme Found in *Escherichia Coli*

It has been reported that diarrhoea can be caused as a result of *Escherichia coli* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Escherichia coli* bacteria. It has been

found that Shikimate dehydrogenase enzyme (protein database code 1NYT) is involved in Phenylalanine, tyrosine and tryptophan biosynthesis (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the Galangal plant were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov). The protein database code of the Shikimate dehydrogenase enzyme was identified from the website (www.rcsb.org). The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER ENERGY" and "-CDOCKER INTERACTION ENERGY" were used as an indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Shikimate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on (a) high positive value of -CDOCKER energy and (b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019, 56(2), 111-121).

In Table-1 it is found that interaction between all the phytochemicals and the enzyme shows negative —CDocker energy and positive —CDocker interaction energy. Thus the results indicated that β-farnesene, α-fenchyl acetate, β-bisabolene, β-bergamotene, β-pinene are least effective in deactivating the Shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Escherichia coli* to small extent.

CONCLUSION

It was previously known that Galangal plant has medicinal action against Diarrhoea. Diarrhoea is caused by *Escherichia coli*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (β-farnesene, α-fenchyl acetate, β-bisabolene, β-bergamotene, β-pinene), which can have a significant interaction with the vital enzyme (Shikimate dehydrogenase) of the microbe. It was found that none of the phytochemicals can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. The phytochemicals of Galangal plant were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of β-farnesene, α fenchyl acetate, β-bisabolene, β-bergamotene, β-pinene provided less medicinal values to Galangal against Diarrhoea caused by *Escherichia coli*.

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Table 1. Results of CDocking of phytochemicals with Shikimate dehydrogenase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN-C DOCKER INTERACTION ENERGY AND –C DOCKER ENERGY
1	β-Pinene	-9.40075	11.4708	20.87155
2	α-fenchyl acetate	-27.849	16.1401	43.9891
3	β-bergamotene	-23.4634	23.3615	46.8249
4	β-farnesene	-36.3945	22.0579	58.4524
5	β-bisabolene	-43.6791	20.8784	64.5575

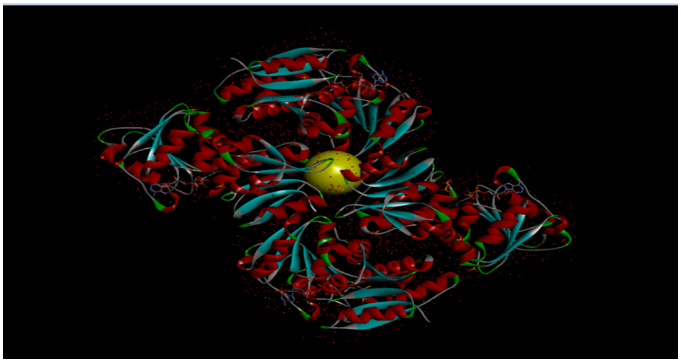

Figure 1. Active site of Shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Kapoor kachri* against Alcohol Dehydrogenase of *Helicobacter pylori* Causing Ulcer

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Kapoor kachri* extract is used to cure Ulcer. The plant extract contains different phytochemicals. Ulcer is caused by *Helicobacter pylori.* One of the key enzymes involved in its biochemical pathway is Alcohol dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive values for both the parameters indicate Lupeol-1 can effectively deactivate Alcohol dehydrogenase enzyme thereby interrupting the life cycle of Helicobacter Pylori.

Key Words: Phytochemical, Biovia, Discovery studio, *Kapoor kachri, Helicobacter pylori*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals ,which can be used for therapeutic purpose.Phytochemicals(From the Greek word Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers,

roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Kapoor kachri is a genus of flowering plants in the family Zingiberaceae and itinvolves chemical family like ketones and oxides. It is a smallest hardy ginger that grows to around 1m, with green leaves and white Flowering plant. The root stalk is useful in local inflammations, nausea, asthma, bronchitis and in pain etc. The rhizome of plant is said to be carminative, stimulant and a tonic. It has been described as useful, specially as an antiasthmatic agent. Clinical trials have been conducted in tropical eosinophilia with promising results. It counteracts had mouth test and smell. It is also known as spiked Ginger Lily. It is called Sandharlika/ kapurkachri in Hindi and Takhellei in manipuri. Common names for plants in the genus vary widely according to region.In this article, we look at the symptoms, causes, risk factors, and possible complications of Ulcers. We also cover when to see a doctor, diagnosis, treatment, and lifestyle and dietary changes. *H. pylori* bacterial infection is the most common cause of Ulcers. During ulcer the blood will be float by rupturing vein and artery which is very painful. Many people first become infected during childhood, but not everyone experiences symptoms. Researchers think *Helicobacter pylori* spreads through infected food, water, salvia and other bodily fluids. This study focuses on the identification of phytochemiclas of Kapoor kachriresponsible to cure Ulcer caused by *Helicobacter pylori.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc., When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Lupeol-1, Ethyl ferulate, 3 hydroxy Gamma eudesmal ,9 hydro hedychenone, Ethyl p Methoxycinnamate, Ethyl cinnamate 1, Gamma Eudesma and Borneol 1 etc. It has already been established that *Kapoor kachri* belonging to family Zingiberaceae has potential to help controlling the Ulcer. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Ulcer

Enzyme Found in *Helicobacter pylori*

It has been reported that chronic gastritis can cause as a result of *helicobacter pylori* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Helicobacter pylori* bacteria. It has beenfound thatAlcohol dehydrogenase (NADP+) (has protein data base of 3PHJ) has a major role in metabolic pathway of Helicobacter pylori as glycine, serine and threoninemetabolism. This enzyme (GbsB) is helps in the conversation of Chlointo Betaine aldehyde which is very crucial for survival of *Helicobacter pylori* (found from KEGG).This enzyme is very abundant enzyme in cytoplasm of *Helicobacter pylori* i.e about 0.5% .

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Kapoor kachri* were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the Alcohol dehydrogenase was identified from the website (https:www.rcsb.org/structure/3TWO). The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Alcohol dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid.

Table 1 shows that shikimate dehydrogenase interaction has the highest positive value of -CDOCKER energy (28.856) and minimum value of the difference (2.7204) between - C DOCKER interaction energy and - C DOCKER energy followed Ethyl ferulate. Thus the results indicated that the Lupeol-1 and can effectively deactivate the Alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *helicobacter pylori.* Here Gamma Eudesma and Borneol 1 found to be very less effective with Alcohol dehydrogenase and Alpha carotene cannot interact with Alcohol dehydrogenase.Thus the key phytochemicals preventing ulcer caused by helicobacter pylori are Lupeol-1 and Ethyl ferulate.

CONCLUSION

It was previously known that *Kapoor kachri* plant has medicinal action against Ulcer caused by *helicobacter pylori.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Lupeol-1, Ethyl ferulate, 3 hydroxy gamma eudesmal, 9 hydro hedychenone, Ethyl p Methoxycinnamate and Ethyl cinnamate 1 etc) which can have a significant interaction with the vital enzyme (Alcohol dehydrogenase (NADP+)) of the microbe. It was found that Lupeol-1 can form better bond in comparison to other phytochemicals like Gamma Eudesma and Borneol 1 were found to be very less effective in deactivating the Alcohol dehydrogenase (NADP+) enzyme.Thus, this study could explain that the presence of the phytochemicals like Lupeol-1 and Ethyl ferulateetc provides the medicinal values to the Kapoor kachri against ulcer caused by helicobacter pylori.

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Table 1. Results of Cdocking of Phytochemicals with Alcohol dehydrogenase(Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Lupeol-1	28.856	31.5764	2.7204
2	Ethyl ferulate	10.0496	12.2011	2.1515
3	3 hydroxygamma eudesmal	13.1128	14.7927	1.6793
4	Ethyl p Methoxycinnamate	22.1193	24.0983	1.979
5	Gamma Eudesma	-32.6517	29.5179	62.1619
6	Borneol 1	-35.8348	18.1646	53.9994

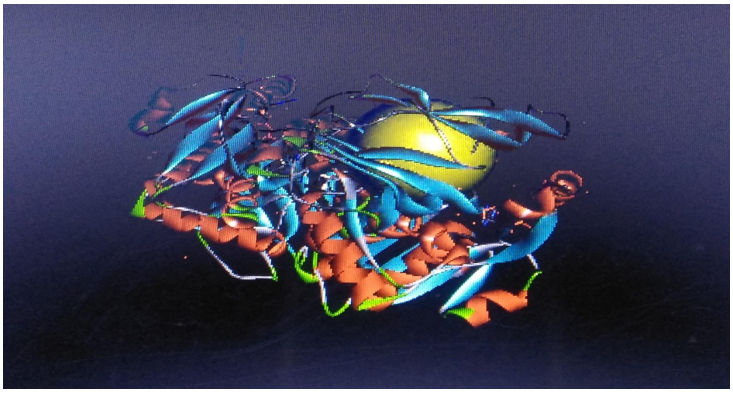

Figure 1. Active Site of Alcohol dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Nardostachys jatamansi* against Ribitol-5-Phosphate-2-Dehydrgenase (NADP+) of *Streptococcus pneumoniae* Causing Pneumonia

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Nardostachys jatamansi* plant extract is used to cure pneumonia. The plant extract contains different phytochemical is caused by *Nardostachys sp.* One of the key enzymes involved in its biochemical pathway is ribitol-5-phosphate-2-dehydrgenase (NADP+) enzyme. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on –Cdocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals some can effectively deactivate the ribitol-5-phosphate-2- dehydrgenase ($NADP^+$) enzyme thereby interrupting the life cycle of *Streptococcus pneumoniae*.

Key Words: phytochemical, Biovia, Discovery studio, *Nardostachys jatamansi, Streptococcus pneumoniae*.

INTRODUCTION

Pneumonia is a form of acute respiratory tract infection (ARTI) that affects the lungs. Pneumonia has many possible causes, but the most common are bacteria and viruses. The most common pathogens are *Streptococcus pneumoniae, Haemophilus influenzae* type b (Hib), and respiratory syncytial virus (RSV) [1], [2]. Pneumonia is the single leading cause of mortality in children under five and is a major cause of child mortality in every region of the world, with most deaths occurring in Sub Saharan Africa and South Asia [3]. In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and

unhealthy.A healthy lifestyle is one which helps to keep and improve people's health and well-being [4]. Many governments and non-governmental organizations work at promoting healthy lifestyles [5]. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (Heinrich Metal.,2010) [6]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K.,2014) [7]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011) [8]. Medicinal plants play a key role in human health care. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Medicinal ethnobotany indigenous peoples use a wide range of plants therapeutically to maintain their health. There is the great discovery of designing drugs that will be most useful for the human. Before getting towards the production of newly design drugs, the importance of the local and indigenous knowledge, practices and innovation should be known. *Nardostachys jatamansi* belongs to family caprifoliaceae. *Nardostachys jatamansi* is a flowering plant of the honey suckle family that grows in the eastern Himalayas, primarily in a belt through Kumaon, Nepal, Sikkim and Bhutan [9]. The plant grows to about 1 m in height and has pink, bell-shaped flowers. It is found at an altitude of 3,000–5,000 m (9,800–16,400 ft). Nard oil is used as a perfume, an incense, a sedative, and an herbal medicine said to fight insomnia, birth difficulties, and other minor ailments [10]. *Jatamansi* leaves extract is used to cure disease like *pneumonia*. *Jatamansi* is known to contain phytochemicals like 1-Octacosanol, Oleanolic acid, β sitosterol, Ursolic acid [11]. There is high possibility that these phytochemicals play a major role in curing *pneumonia*. However, there is no report identifying the specific phytochemical responsible to cure *pneumonia*.

A group of bacteria belonging to order *Streptococcaceae* generally cause *pneumonia* .This is a type of atypical bacteria that commonly causes mild infection to the respiratory system. *Streptococcus pneumoniae* frequently colonizes the upper respiratory tract. The human nasopharynx is the only natural reservoir for S. pneumoniae and these bacteria along with viruses are commonly found in a child's nose or throat; these pathogens are then aspirated into the lungs, causing disease. Pneumonia can be spread in a number of ways. The pathogen is transmitted through direct contact with respiratory secretions, colonizes the nasopharynx and may then cause blood-borne diseases[12].

Pneumonia is an inflammatory condition of the lung affecting primarily the small air sacs known as *alveoli* (McLuckie,A.,ed,2009)[13](Leach,Richard E.,2009) [14]. Typically symptoms include some combination of productive or dry cough, chest pain, fever, and trouble breathing (Ashby B, Turkington C,2007) [15]. *Pneumonia* is usually caused by the bacteria or viruses and less commonly by other microorganisms, certain medications and condition such as autoimmune diseases (McLuckie,A.,ed,2009)[12] (Jeffrey C.Pommerville,2010) [16]. This study focuses on the

identification of the phytochemical of *Nardostachys jatamansi* responsible to cure pneumonia caused by *Streptococcus pneumonia.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that jatamansicontains 1-Octacosanol, Oleanolic acid, β sitosterol, Ursolic acid etc. It has already been established that *Nardostachys jatamansi* plant belonging to caprifoliaceae family has potential to help controlling pneumonia. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling ofpneumonia.

Enzyme Found in *Streptococcus pneumoniae*

It has been reported that pneumonia can cause as a result of *Streptococcus sp* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Streptococcus pneumoniae* bacteria. It has been found that is ribitol-5-phosphate-2-dehydrgenase (NADP+)(protein database code 2VSI) is involved in lipid metabolism, palmitate biosynthesis,arachidnate biosynthesispathway (BRENDA) and very crucial for survival of the particular microbe[17].

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Nardostachys sp* plant were downloaded from the website [18]. The protein database code of is ribitol-5-phosphate-2-dehydrgenase(NADP+) enzyme was identified from the website [19]. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under “receptor-ligand interaction”.The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the ribitol-5-phosphate-2-dehydrgenase ($NADP^+$) enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic

methods. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the non-bonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019, 56(2), 111-121) [20].

Table 1 shows that ribitol-5-phosphate-2-dehydrgenase ($NADP^+$)-1-Octacosanolinteraction has the highest positive value of -CDOCKER energy (50.7985) and minimum value of the difference (6.4608) between - C DOCKER interaction energy and - C DOCKER energy followed by β sitosterol. Thus the results indicated that 1-Octacosanolcan effectively deactivate the ribitol-5-phosphate-2-dehydrgenase ($NADP^+$)- enzyme thereby interrupting the biological cycle of *Nardostachys jatamansi*. Higher positive values for 1-Octacosanol indicated that it was the most active ingredient against *Nardostachys sp*. On the other hand, Oleanolic acid, β sitosterol , Ursolic acid can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy).Thus, the key phytochemicals preventing pneumonia caused by *Nardostachys sp.*is 1-Octacosanol.

CONCLUSION

It was previously known that *Nardostachys jatamansi* plant has medicinal action against pneumonia. Pneumonia is caused by *Streptococcus pneumoniae*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (1-Octacosanol, Oleanolic acid, β sitosterol, Ursolic acid), which can have a significant interaction with the vital enzyme ribitol-5-phosphate-2-dehydrgenase (NADP+) of the microbe. It was found that 1-Octacosanol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Oleanolic acid, β sitosterol, Ursolic acid is found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of 1-Octacosanolprovided the medicinal values to *Nardostachys jatamansi* against cholera caused by *Streptococcus pneumoniae*.

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Table 1:Results of Cdocking of Phytochemicals with Ribitol-5-Phosphate-2-Dehydrgenase(NADP+) (Receptor)

Sl No	Ligand	-Cdocker Energy	-Cdocker Interaction Energy	Difference Between –Cdocker Interaction Energy And -Cdocker Energy
1.	1-Octacosanol	50.7985	57.2593	6.4608
2.	β sitosterol	-29.939	46.361	76.3
3.	Oleanolic acid	-49.8437	37.0717	86.9154
4.	Ursolic acid	-42.819	51.9525	94.7715

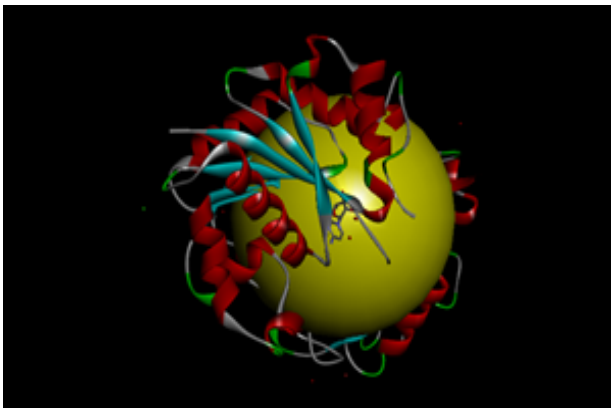

Fig.1. Image of Receptor Ligand Interaction

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Theobroma cacao* against Alcohol Dehydrogenase of *Entamoeba histolytica* sp. causing Dysentery

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ABSTRACT

Phytochemicals are non-nutritive substances that can be obtained from plants. *Theobroma cacao* contains various phytochemicals which can be used to cure dysentery.*Theobroma cacao*, an economically important tropical-fruit tree crop that is the source of chocolate. Cocoa is unusually rich in phytochemicals. In dried and unfermented cocoa beans, pigment cells make up about 11e13% of the tissue.Consuming chocolate has been reported to increase the total antioxidant capacity in human blood plasma These cells are rich in polyphenolic compounds, in particular catechins, although the characteristic purple color of cocoa beans is due to anthocyanins.*Salmonella sp*. cause dysentery. Alcohol dehydrogenase is one of the important enzymes involved in biochemical pathway. BIOVIA discovery studio was used to study the molecular docking of phytochemical. Based on –CDOCKER energy and –CDOCKER interaction was evaluated. Out of different phytochemicals quercetin, Chlorogenic acid, phloretic acid, iso-orientin, vanillic acid and caffeine can effectively deactivate alcohol dehydrogenase enzyme thus interrupting the life cycle of *Entamoeba histolytica.*

Keywords: - phytochemical, BIOVIA, Discovery studio, *Theobroma cacao, Entamoeba histolytica.*

INTRODUCTION

In older days, life was natural and though it was slow and difficult at time. Today, in modern time thought life is fast paced, comfortable, readymade it is also stressful and unhealthy. In recent years, the interest in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide [1]. Some dangerous health hazards like blood pressure, diabetes, obesity etcare exposed to is due to over changing mark conditions, unhealthy eating habits, less physical exhaustion, comfortable and stressful life. Various medicinal agents can be found in natural and modern drugs also have been derived from natural sources. *Theobroma cacao* L. contains more than different chemical compounds some of which have been traditionally used for their anti-oxidant, antimicrobial activity [2]. It has long been recognized as an important health problem in developing countries and global threat to human health [3]. This study performed some phytochemical characterizations. It has confirmed that cacao leaves consist some of bioactives compounds which are potential for further activities in pharmaceutical disease. Traditional medicine includes all kinds of folk medicine unconventional medicine and indeed any kind of therapeutic method that had been handed down by the traditional of a community or ethinic group [4]. The chemical substances present in the plants a definite physiological action on human body are call phytochemicals. These phytochemicals can be used for therapentic purposes. They can be derived from any part of the plant like bark, leaves, flower, fruits and seeds. The phytochemicals have various medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbialetc. The presence of important phytochemicals andanti-oxidants in the leaves is a scientific justification of the traditional use of the plant in folklore medicine against various diseases [5]. Phytochemicals analysis of these plants reveals that some plants are promising sources of chemical Constitution like alkaline, flavonoids, glycosides, phenolic, phenol and antioxidant with free radical scavenging activity in varying proportions and possess anti- diarrhoeal activity [6]. They played an important role in preparation of allopathic, homeopathic, aromatherapy etcand for various pharmaceutical products. Medicinal plants are the origin of various drugs for modern medicinal system for ex- the herb foxglove is the source of digital is and the herb salicin is the source for aspirin. The breast cancer fighting drug texol comes from specific yet tree etc. About 80%of the world population depends on traditional medicine. The traditional medicine is chiefly plant based. They are popular because they are safe, efficient and cost effective. Phytochemicals serve as starting materials for a member old and new pharmaceutical product. Aabout 25%of modern pharmaceutical drugs have originated from plants.

Cocoa belongs to family malvaceae. Cocoa extract is used to cure disease like dysentery. *Theobroma cacao* also called the cocoa tree and the cocoa plant is a small evergreen tree native to the deep tropical regions of mesoamerica. Cocoa leaves extract is cure disease like dysentery. Cocoa is known to contain phytochemicals like procyanidin,procyanidin B1,B3,B4,C1,D, pruning, querertin, syringing acid, Theobromine, vitexin, venilic acid, caffenine , chorogenic acid , iso-orientin , hyperin, feralic acid, iso quercetin, isovitexines, naringenin, leutotin, orentin, p-coumaric acid, phenylacetic acid, phloretic acidetc.There is high possibility that these phytochemicals play a major role in curing dysentery. However, there is no report identifying the specific phytochemical responsible to cure dysentery.

A group of bacteria belonging to genus *Shigella* generally causes dysentery. They are rod-shaped Gram-negative bacteria. *Shigella* infection (shigellosis) is a common bacterial disease that affects the intestinal tract. *Shigella* bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food. This study focuses on the identification of the phytochemical of *Theobroma cacao* responsible to cure dysentery caused by *Entamoeba histolytica sp.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Thiobroma cacao* contains procyanidin, procyanidin B1, B3, B4, C1, D, pruning, querertin, syringing acid, Theobromine, vitexin, vanilic acid, caffenine, chorogenic acid, iso-orientin, hyperin, feralic acid, isoquercetin, isovitexines, naringenin, leutotin, orentin, p-coumaric acid, phenylacetic acid, phloretic acid etc. It has already been established that *Thiobroma cacao* plant belonging to Malvaceae family has potential to help controlling dysentery. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of dysentery.

Enzyme found in *Entamoeba histolytica*

It has been reported that dysentery can causeas a result of *Entamoeba histolytica sp.* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Entamoeba histolytica sp.* bacteria. It has been found that alcohol dehydrogenese enzyme (protein database code1Y9A) is involved inmethionine metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Thiobroma cacao* plant were downloaded from the website. The protein database code of the alcohol dehydrogenase enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the -CDocker protocol of BIOVIA softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the alcohol dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of –CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019, 56 (2), 111-121). Table 1 shows that alcohol dehydrogenase-quercetin interaction has the highest positive value of -CDOCKER energy (23.5611) and minimum

value of the difference (2.3666) between - C DOCKER interaction energy and - C DOCKER energy followed by chlorogenic acid. Thus, the results indicated that Quercetin, chlorogenic acid, phloretic acid, iso-orientin, vanillic acid and caffeinecan effectively activate the alcohol dehydrogenase enzyme there by interrupting the biological cycle of *Entamoeba histolytica*. Higher positive values for Quercetin indicated that it was the most active ingredient against *Entamoeba histolytica.* Thus, the key phytochemicals preventing dysentery caused by *Entamoeba histolytica* are Quercetin, chlorogenic acid, phloretic acid, iso- orientin, vanillic acid and caffeine.

CONCLUSION

It was already known that *Theobroma cocoa* plant is helpful in diarrhea. *Salmonella sp***.** cause dysentery. This study represent the theoretical basis of this observation. Molecular docking operation was carried out using studio module of BIOVIA software to identify phytochemicals procyanidin,procyanidin B1,B3,B4,C1,D, pruning, querertin, syringing acid, Theobromine, vitexin, vanilic acid, caffenine, chorogenic acid, iso-orientin, hyperin, feralic acid, isoquercetin, isovitexines, naringenin, leutotin, orentin, p-coumaric acid, phenylacetic acid, phloretic acid etc. which can interact significantly with vital enzyme alcohol dehydrogenase of the microbe. Quercetin and chlorogenic acid sucssecfully inhibit the metabolic cycle of the microbe as it forms strong bond with the enzyme. It was found that phloretic acid, iso- orientin, vanillic acid and caffeine activate the enzyme of the microbe effectively. Thus, from this study it can be concluded that the presence of quercetin, chlorogenic acid, phloretic acid, iso-orientin, vanillic acid and caffeine in *Theobroma cocoa* provide medicinal value again dysentery which is caused by *Salmonella*.

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Table 1. Results of C Docking of phytochemicals with alcohol dehydrogenase receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Quercetin	23.5611	25.9277	2.3666
2	Chlorogenic acid	20.8964	37.8669	16.9705
3	Phloretic acid	19.7742	19.3759	-0. 3983
4	Iso-orientin	18.0565	39.9249	21.8664
5	Vanillic acid	15.3883	16.7168	1.3285
6	Caffeine	11.1851	19.5276	8.3425

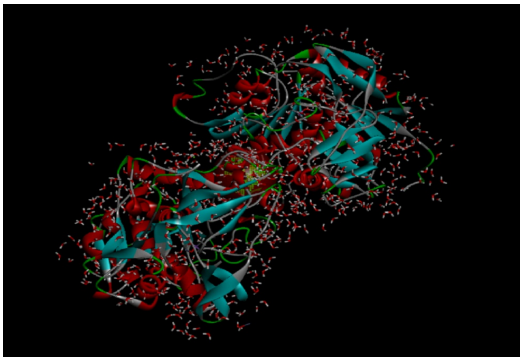

Figure 1. Active site of alcohol dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Withania somnifera* against Acnes Vulgaris Caused by *Propionibacterium acnes*

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ABSTRACT

Phytochemicals are naturally found in plants and are responsible for providing color, flavor, and aroma to fruits and vegetables. They are biologically active and function to protect plants against invasion, disease, and infection. Acne vulgaris is a disease of pilosebaceous unit which is characterized by non-inflammatory and inflammatory lesions. Common therapies that are used for the treatment of acne include topical, systemic, hormonal, herbal and combination therapy. So, for the treatment of acne herbal anti acne cream *Withania somnifera* extract has been used from the ancient times due to its anti-inflammatory action and antioxidant property. Herbal medications are considered safer than allopathic medicines because allopathic medications are associated with different side-effects such as allergy, local irritation, scaling, photosensitivity reaction, itching, peeling, redness etc. In this study, one of the key enzyme involved in the biochemical pathway of *Propionibacterium acnes* is D-lactate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied & carried out using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals cuscohygrine and anahygrine can effectively deactivate the D-lactate dehydrogenase enzyme thereby interrupting the life cycle of *Propionibacterium acnes*.

Keywords: - Phytochemical, Biovia, Discovery studio, *Withania somnifera, Propionibacterium acnes*

INTRODUCTION

Skin is the most vulnerable part of our body. In day to day life, exposure to sun and dust leads to number of problems such as pimples, acne, sunburn marks and pigmentation (Mullaicharam and Elahadi, 2012; Kapoor and Swarnalata, 2011). Acne vulgaris is a disease of pilosebaceous glands which is characterized by non-inflammatory (open and closed comedones) and inflammatory lesions (papules, pustules and nodes). The conditions usually start at the age of 14 to 19 years. A change in keratinisation pattern of hair follicle leads to blockage of sebum secretion (Tare et al. 2013; Tahir, 2010). It is hypersensitivity to the stimulation of sebocytes and follicular keratinocytes by androgen leads to hyperplasia of sebaceous glands and seborrhea which characterize acne.

The most common bacteria responsible for acne is *Propionibacterium acnes* and have been isolated from acne patients. The *P. acnes* colonize the follicular duct and proliferates, which results in the conversion of sebum into triglycerides which are probably causing inflammation. The use of natural remedies as well as herbal medication is in practice since thousand years. With minor side-effects and much advantages of multi functionality, the herbal medicines are being used in different formulation to cure many diseases (Ray, 2013).

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Indian Ginseng is a valuable medicinal plant used since ancient times (Winters, 2006). It is an evergreen shrub belonging to the solanaceae family. It is widely used in traditional system of medicine in India (Unani and Ayurvedic system). *W. somnifera* grows in most parts of the world mainly in tropical and subtropical zones. However, it is abudnatly found in sub-Himalayan (1000 m) tracts in India, Pakistan, Sri Lanka, Afghanistan, South Africa Jordan, Egypt and Morocco. Among the 23 species of Withania genus, this plant is economically more valuable because of its medicinal importance (Mirjalili 2009a; Kulkarni and Dhir 2008). Various pharmacological activities have been documented to be associated with *W. somnifera.* It is regarded as “Rasayana” in Ayurveda which means potent rejuvenator, as it enhances haemoglobin count, hair melanin pigmentation (Singh et al. 2011) physiological endurance and general health (Dhar et al. 2012). All parts of this plant have been reported to possess medicinal values e.g. its roots are used to treat various physiological disorders. Their paste or powder is used to cure ulcers, rheumatic swellings and as a nutrient or health restorative in pregnant or old age people. Similarly, the leaves of this plant are used as antihelmantic, anti-pyretic, anti-cancer, anti-tuberculosis and anti-ulcer. Furthermore, various parts of this plant are constituents of more than 200 formulations in Indian system of traditional medicine and have been used to cure various health disorders (Gauttam and Kalia 2013; Sukanya et al. 2010). Ashwagandha is known to contain phytochemicals like Anahygrine, pseudopelletierine, cuscohygrine etc. (NCBI). Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.This study focuses on the identification of the phytochemical of *Withnia somnifera* responsible to cure pimples caused by *Propionibacterium acnes*.

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software, Dassault Systemes of France was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

Phytochemical Used

W. somnifera contains steroidal compounds, including anahygrine, pseudopelletierine, cuscohygrine etc. (NCBI). It has already been established that *W. somnifera* extracts has potential to help controlling peptic ulcer. The sdf files for the phytochemicals found in the *W. somnifera* plant were downloaded from the website PUBCHEM.

Enzyme Used

It has been reported that pimples can formed as a result of *P. acnes* infections. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *P. acnes* bacteria. It has been found that D-lactate dehydrogenase enzyme (PDB ID- 2DLD) is involved in metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. The protein database code of the D-lactate dehydrogenase enzyme was identified from the RCSB PDB . The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

The active site of the D-lactate dehydrogenase enzyme has been presented in Figure1. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy.

It has been indicated that the interaction has the highest positive value of -CDOCKER energy, 25.00 and minimum value of the difference, 10.075 between - C DOCKER interaction energy and - C DOCKER energy (Table 1). Thus the results indicated that cuscohygrine can effectively deactivate the D-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *P. acnes* in comparision to the other phytochemicals such as Anahygrine and Pseudopelletierine. Anahygrine is moderately effective against D-lactate dehydrogenase enzyme.

CONCLUSION

From the above study it has been concluded that using Discovery studio module of BIOVIA software, molecular docking operation result indicated that the phytochemical, cuscohygrine have an effective interaction with the vital enzyme, D-lactate dehydrogenase enzyme of the *P. acnes*. It was found that cuscohygrine can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of cuscohygrine acid provided the medicinal values to *W. somnifera* against acnes vulgaris caused by *P. acnes.*

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Table 1. Results of CDocking of phytochemicals with glycerol dehydrogenase (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Anahygrine	8.76	28.17	19.41
2	Pseudopelletierine	-26.45	21.86	48.35
3	Cuscohygrine	25.00	35.08	10.075

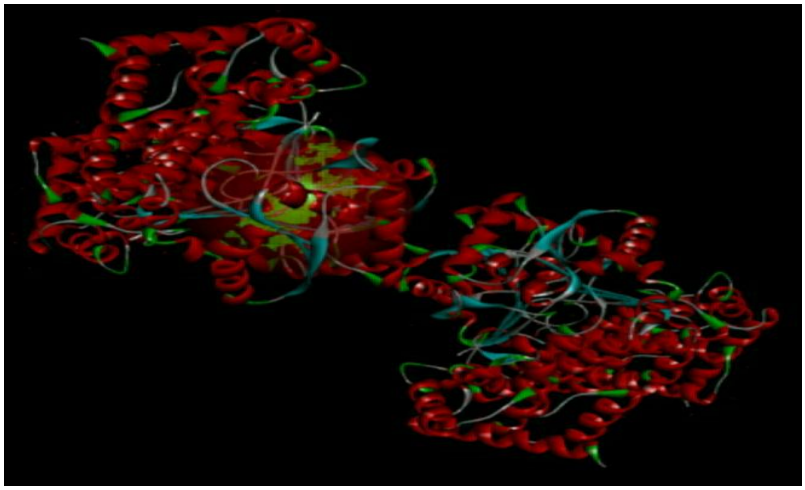

Figure 1. Active site of D-lactate dehydrogenase enzyme.

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RESEARCH ARTICLE

An *In-silico* Investigation of Phytochemicals from *Jasminum grandiflorum (*Jasmine*)* as Anti-Viral Agent against Hepatitis

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ABSTRACT

Phytochemicals, as plant components with discrete bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to provide health benefits. It is important to establish the scientific rationale to defend their use in foods, as potential nutritionally active ingredients. Phytochemicals could provide health benefits as, substrates for biochemical reactions, cofactors of enzymatic reactions, inhibitors of enzymatic Hepatitis can be cured by Jasmine. Hepatitis is caused by *Hepatitis A.* One of the key enzymes involved in its biochemical pathway is Triglyceride lipase. The molecular docking of the phytochemicals with the enzyme was studied using BIOVIA Discovery Studio. The strength of the interaction was evaluated based on -C DOCKER energy and -C DOCKER interaction energy.

Keywords: - phytochemical, BIOVIA, Discovery studio, *Jasminum, Hepatitis A*

INTRODUCTION

In ancient time there was no toxic environment, air, or water. There were beautiful nature and the people were good to nature also. But now-a-days there is huge population growth, high demands of food and many more basic needs. As there is less time and more demands, so we create artificial way to fulfill our demands. These reasons affect the nature, the environment and our life style also. This changing lifestyle causes more diseases like diabetes, blood

pressure, heart diseases, constipation, joint pains, and many more. No doubt we are going again the nature only for our demands but still there is remedies for many diseases from nature.

Consuming a diet rich in plant foods will provide a milieu of phytochemicals, nonnutritive substances in plants that possess health-protective benefits. Nuts, whole grains, fruits, and vegetables contain an abundance of phenolic compounds, terpenoids, pigments, and other natural antioxidants that have been associated with protection from and/or treatment of chronic disease such as heart disease, cancer, diabetes, and hypertension as well as other medical conditions (Winston J Craig,1997). Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. Medicinal plants play a key role in human health care. Plants are considered as one of the most important sources of medicines. Among the 2, 50,000 higher plant species reported in the world, more than 80,000 species are being used as medicinal. The medicinal plants are extensively utilized throughout the world and are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of world population(Sudhanshu Kumar Jain1968). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy.drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain(Marcy J Balunas,2005).

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug Taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Estimating the incidence of waterborne infectious disease related to drinking water in the United States(OA Fawole, 2010).

Jasmine belongs to family oleaceae. *Jasminum grandiflorum* Linn. is one of such important plants. It has been extensively used by the tribes all over India to treat different diseases which mainly include body pains, toothache, stomach ache, ulcers, and sexual impotency. Jasmine flower extract is used to cure disease like Hepatitis.(Mittal Arun et al, 2016). Ja The hepatitis A virus (HAV), a picornavirus, is a common cause of hepatitis worldwide (Jennifer A Cuthbert,2001). There is high possibility that these phytochemicals play a major role in curing Hepatitis. However, there is no report identifying the specific phytochemical responsible to cure hepatitis.

A group of bacteria belonging to genus hepatitis generally cause hepatitis. Hepatitis infection is a common bacterial disease that affects the intestinal part. *Hepatitis bacteria* typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food. Diseases due to parasites and viruses were estimated based upon the prevalence of specific pathogens or pathogen groups in drinking water coupled with models of infectivity (Robert D Morris, Ronnie Levin, 1995). The hepatitis A virus (HAV), a picornavirus, is a common cause of hepatitis worldwide (Jennifer A Cuthbert, 2001).This study focuses on the identification of the phytochemical of *Jasminum* responsible to cure hepatitis caused by *hepatitis A*.

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. It has already been established that *Jasminum* plant belonging to Oleaceae family has potential to help controlling hepatitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Hepatitis. Typical acute hepatitis was reproduced in a human volunteer immune to *Hepatitis A* virus (HAV) after oral administration of pooled stool extracts from presumed cases of epidemic non-A, non-B hepatitis(MS Balayan, et.al.1983).

Enzyme found in *Hepatitis A*

It has been reported that hepatitis can cause as a result of *Hepatitis A* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Hepatitis A* virus. It has been found that Triglycerol lipase (1LBS) is involved in some metabolic process and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Jasminum* plant were downloaded from the website. The protein database code of the Triglycerol lipase enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the C DOCKER protocol of BIOVIAsoftware under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-C DOCKER_ENERGY” and “-C DOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Triglycerol lipase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-C DOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -C DOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on high positive value of -C DOCKER energy and small difference between -C DOCKER energy and -C DOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala). Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019, 56(2), 111-121). Table 1 show that superoxide reductase-chrysin interaction has the highest positive value of -C DOCKER energy (27.0095) and minimum value of the difference (10.075) between - C DOCKER interaction energy and - C DOCKER energy followed by hesperidin. Thus, the results indicated that dotriacontanol and hesperidin can effectively deactivate the Triglycerol lipase enzyme thereby interrupting the biological cycle of *Hepatitis A*. Higher positive values for dotriacontanol indicated that it was the most active ingredient against *Hepatitis A*. On the other hand, ducosterol, iridodial glycoside, oleanolic acid can deactivate the enzyme to a small extent (negative -C DOCKER energy but positive -C DOCKER interaction energy). Benzyl-6-O-beta-D-xylopyaranoside and 2-phenylethyl beta-primeveroside cannot interact with Triglycerol lipase. Thus, the key phytochemicals preventing hepatitis caused by *Hepatitis A*. are hesperidin, dotriacontanoic acid, dotriacontanol.

CONCLUSION

It was previously known the *Jasminum* plant has medicinal action against Hepatitis. Hepatitis is caused by *Hepatitis A*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical (ducosterol,hesperidin, dotriacotanoic, iridodial glycosides, oleanolic acid), which can have a significant interaction with the vital enzyme (superoxide reductase) of the microbe. It was found that alkaloids and flavonoids can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Oleanolic acid, ducosterol and iridodial glycoside were found to be not much effective in deactivating the enzyme of the microbe. Benzyl-6-O-beta-D-xylopyaranoside and 2-phenylethyl beta-primeveroside cannot deactivate the enzyme. Thus, this study could explain that the presence of hesperidin, dotriacontanoic acid, do triacontanol provided the medicinal values to *Jasminum* against hepatitis caused by *Hepatitis A*.

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Table 1. Results of CDocking of phytochemicals with Triglycerol lipase(receptor)

SL. NO.	LIGAND	-C DOCKER ENERGY	-CDOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Dotriacontanoic acid	59.2727	61.0123	1.7396
2	Dotriacontanol	61.2625	64.8399	3.5774
3	Hesparidin	10.8623	64.9745	54.1122
4	Daucosterol	-47.9385	52.368	100.3065
5	Iridoidal glycosides	-108.163	68.4203	176.5833
6	Oleanolic acid	-59.8214	42.7897	102.61111
7	Benzyl-6-O-beta-D-xylopyaranoside	Failed	Failed	NA
8	2-phenylethyl beta-primeveroside	Failed	Failed	NA

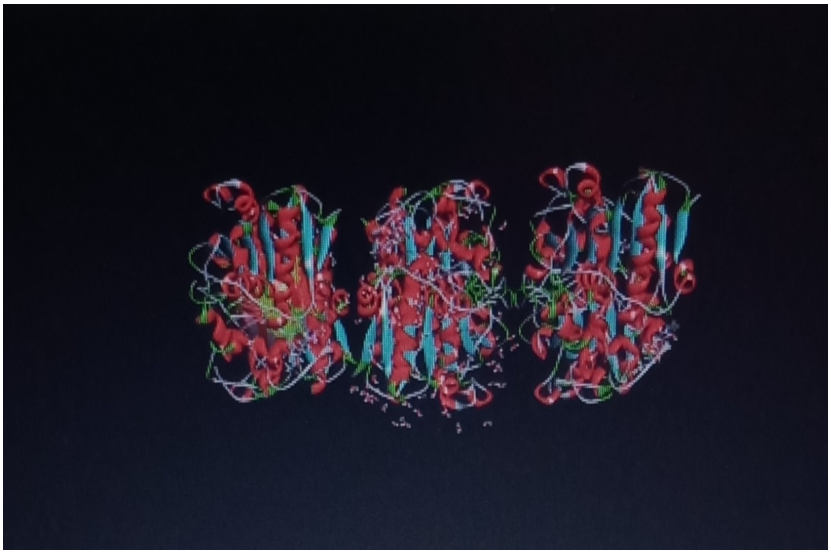

Figure 1. Active site of Triglycerol lipase

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RESEARCH ARTICLE

In silico Molecular Docking Analysis of Phytochemicals from *Syzygium aromaticum* against Infections in Feet Crack Caused by *Trichophyton rubrum*

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ABSTRACT

In past decades the modern lifestyle is leading populations to follow an unhealthy habituations pattern. With the developments of science more and more synthetic drugs from chemicals are being used which are quite effective too. But the downside of these drugs is that they contain side effects to the Individuals on the medication sometime making the matter much worse. (Oxidative stress and membrane permeability as mode of antibacterial activity of aqueous extract of *Syzygium aromaticum* seeds against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* was investigated. The concentration of phytochemical constituents of *Syzygium aromaticum* was determined using gas chromatography.) Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Syzygium aromaticum* plant extract can be used to cure infection in feet cracks. The plant extract contains different phytochemicals. Infections in feet crack is caused by *Trichophyton rubrum*.The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Myricetin can effectively deactivate the microbial metabolic enzyme thereby interrupting the cycle of the infection causing microbes, *Trichophyton rubrum*. *Trichophyton rubrum* is one of the most common causes of chronic tinea

pedis commonly known as athlete's foot. Chronic infections of tinea pedis result in moccasin foot, in which the entire foot forms white scaly patches and infections usually affect both feet.

Keywords: - phytochemical, BIOVIA, Discovery studio, *Syzygium aromaticum, Trichophyton rubrum.*

INTRODUCTION

Lifestyle includes day to day behaviours and functions of individuals in job, activities, fun and diet. In ancient days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards [1]. Moreover, high production rate of synthesised dugs and easy availability leads todays populations to fall into habit of intaking of these drugs. But with course of time it has been remarked that these drugs cause some adverse side effects on the individual which are far more harmful and fatal in nature [2, 3]. Mean while, Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs has been derived from natural source [4]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds[5]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action, etc[6]. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products while proving to be safe of side effects and cost effective [8].

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models [7]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Phytochemical constituents are derived from medicinal plants with antimicrobial activity. Several medicinal plants extracts have been screened for bactericidal activity with *Syzygium aromaticum* featuring prominently as good antimicrobial plant. *Syzygium aromaticum* (Syn. *Caryophyllus aromaticus*, *Eugenia aromatica, E. caryophyllata*) commonly known as clove tree belongs to the family Myrtaceae which is a pyramidal or conical evergreen tree, about 9-12 meter high. The plant has numerous medicinal properties. The flower buds (cloves) are carminative, stimulant and antimalarial. It is used in dyspepsia, gastric trouble, nausea and vomiting. Its oil is a strong germicide, antiseptic, analgesic, local anesthetic, antioxidant, emetic and spasmolytic. Clove belongs to family Myrtaceae. The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green and then transition to a bright red when ready for harvest. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. Cloves are available throughout the year due to different harvest seasons in different countries.Clove leaves extract is suspected to be used in curing disease like Feet crack infection. Clove is known to contain phytochemicals like Beta-caryophyllene, Compesterol 3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillin, etc[9, 10].There is high possibility that these phytochemicals play a major role in curing the infections. However, there is no report identifying the specific phytochemical responsible to cure the disease. A

group of fungus belonging to familyArthrodermataceae generally cause infection in feet crack. *Trichophyton rubrum* is a dermatophytic fungus of the phylum *Ascomycota*. It is an exclusively clonal, anthropophilic saprotroph that inhabits the upper layers of dead skin, and is the most common origin of foot infections worldwide [11]. It is a common skin infection of the feet caused by fungus. Symptoms often include itching, scaling, cracking and redness. In worst cases the skin may blister. Most often it grows between the toes. The next most common area is the bottom of the foot. The same fungus may also affect the nails or the hands. The infection is typically developed by coming into contact with infected skin, or fungus in the environment [12]. Moisture, sweat, tight-fitted shoes encourages the infection. Nevertheless, Laccase is a decisive enzyme which is responsible foroxidation of phenolic and aminophenol compounds for the fungus [13]. If the enzyme is inhibited the disease may be possibly cured by controlling the metabolic activities of the microbe. This study focuses on the identification of the phytochemical of *Syzygium aromaticum* responsible to cure infection in feet cracks caused by *Trichophyton.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these Phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Syzygium aromaticum* contains Beta-caryophyllene, Compesterol 3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillin, etc. It has already been established that *Syzygium aromaticum* plant belonging to Myrtaceae family has potential to help controlling the infection. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of the infection in feet cracks.

Enzyme found in *Trichophyton rubrum*

It has been reported that infection in feet crack can cause as a result of *Trichophyton rubrum* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Trichophyton rubrum*. It has been found that Laccaseenzyme (protein database code 1KYA) is involved in oxidation of phenolic and non-phenolic compound for the fungus and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Syzygium aromaticum* plant were downloaded from the website [14]. The protein database code of the Laccase enzyme was identified from the website [15]. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-

CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Laccase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy.

Table:1 shows that (Myricetin)-(Laccase) interaction has the highest positive value of -CDOCKER energy(30.7642) and minimum value of the difference (5.6837) between - C DOCKER interaction energy and - C DOCKER energy along with vanillin. Thus, the results indicated that Myricetin can effectively deactivate the Laccase enzyme thereby interrupting the biological cycle of *Trichophyton rubrum*. Higher positive values for Myricetin indicated that it was the most active ingredient against Laccase followed by Rhamnetin, Gallic acid,Kaempferol, Vanillin and Eugenol. On the other hand, Beta-caryophyllene, Stigmasterol, Compesterol 3-beta-d-glucoside, Oleanoid acid can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemical preventing Oral infections caused by *Trichophyton rubrum*is Myricetin.

CONCLUSION

It was previously known that *Syzygium aromaticum* plant has medicinal action against Infections in Oral cavity. This infection is caused by *Trichophyton rubrum*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Beta-caryophyllene, Compesterol 3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillin), which can have a significant interaction with the vital enzyme Laccase of the microbe. It was found that Myricetincan and vanillin form strong bond with the enzyme followed by Rhamnetin, Kaempferol, Gallic acid, Vanillin and Eugenol successfully inhibiting the metabolic cycle of the microbe. While Beta-caryophyllene, Stigmasterol, Compesterol 3-beta-d-glucoside, Oleanoid acid were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Myricetin provided the medicinal values to *Syzygium aromaticum* against Oral infections caused by *Trichophyton rubrum*.

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Table 1. Results of CDocking of phytochemicals with Laccase enzyme (receptor)

SL NO	LIGAND	-C DOCKER ENERGY	-CDOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Myricetin	30.7642	36.4479	5.6837
2	Rhamnetin	25.8526	31.7149	5.8623
3	Gallic acid	24.6676	22.3528	-2.3148
4	Kaempferol	23.2967	28.4838	5.1871
5	vanillin	14.7127	17.7633	3.0506
6	Eugenol	9.43861	20.3223	10.88369
7	Beta-caryophyllene	-19.5183	22.9212	42.4395
8	Stigmasterol	-41.0718	39.2297	80.3015
9	Compesterol 3-beta-d-glucoside	-45.9939	53.1614	99.1553
10	Oleanoid acid	-79.5527	31.1846	110.7373

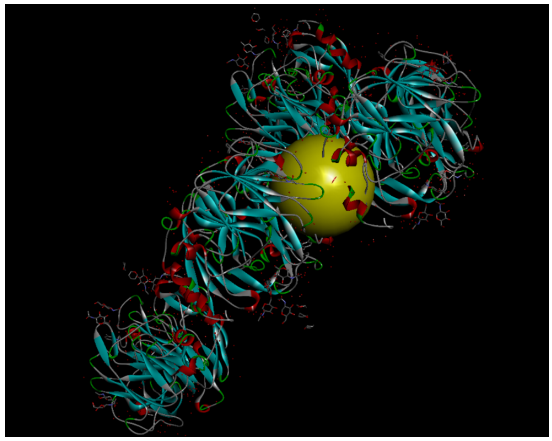

Figure 1. Active site of Laccase enzyme

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals Screened from *Pogostemon cablin* Plant Extract against HlyU Enzyme of *Vibrio cholerae* Causing Cholera

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ABSTRACT

Phytochemicals, as plant components with discrete bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to provide health benefits (Cora J Dillard, 2000). Consuming a diet rich in plant foods will provide a milieu of phytochemicals, nonnutritive substances in plants that possess health-protective benefits (Winston J Craig, 1997). It has been reported that pogostemon cablinplant extract is used to cure cholera. Cholera, caused by *Vibrio cholerae* O1 and O139, is characterized by profuse purging of watery stools, and vomiting and dehydration (Sujit Kumar Bhattacharya, 2003). The plant extract contains different phytochemicals. The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms (Gislene GF Nascimento et al, 2000). Cholera is caused by *Vibrio cholerae.* One of the key enzymes involved in its biochemical pathway is Transcriptional activator HlyU. The molecular docking of the phytochemicals with the enzyme was studied using BIOVIA Discovery Studio. The strength of the interaction was evaluated based on -C DOCKER energy and -C DOCKER interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Tilianin, Retusin, ombuin can effectively deactivate the Transcriptional activator HlyU enzyme thereby interrupting the life cycle of *Vibrio cholera.*

Keywords: - phytochemical, BIOVIA, Discovery studio, *Vibrio cholerae, Pogostemon cablin*.

INTRODUCTION

Human health is precious gift, we all should take care of it. But now-a-days there are negative effects of polluted environment on our health, so we are affected by types of diseases. There are many social and environmental impacts on human health. Diets link environmental and human health. Rising incomes and urbanization are driving a global dietary transition in which traditional diets are replaced by diets higher in refined sugars, refined fats, oils and meats (David Tilman, Michael Clark, 2014). The unhealthy lifestyle and unhealthy surrounding affect our body. External sources like environmental pollution, toxic metals, cigarette smoke, pesticides, etc., which add damage to our body system (G Smilin Bell Aseervatham et al,2013).Due to these unhealthy lifestyles there may develop types of diseases such as heart diseases, diabetes, and many more. Parasitic diseases remain a major public health problem affecting hundreds of millions of people, particularly in tropical developing countries (Senyo Tagboto, Simon Townson, 2001). Nature has already bestowed on us a wide variety of antiviral remedies in the form of herbs, they are characterized with a broad antiviral spectrum (Esmail Al-Snafi Ali, 2015).

Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). The medicinal plants contain several phytochemicals such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals, etc.(S Madhuri, Govind Pandey, 2009).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from Atropa belladonna and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Vibrio cholerae is the causative agent of the diarrheal disease cholera, which is usually acquired by oral ingestion of the bacterium with contaminated water or food. The bacterium expresses virulence factors that allow it to colonize the human intestine and cause the disease. Regulation of virulence genes in *V. cholerae* can involve the ToxR, Fur, or HlyU regulatory system.*Vibrio cholerae* can be considered as the virgin territory in protein chemistry as there is little information available about the proteome; most of the work in *V. cholerae* is confined to the genetic level. Currently, there is almost no information available about HlyU, including its purification. *Pogostemon cablin* belongs to family Lamiaceae (Fabienne Deguerry et al, 2006). Patchouli leaves extract is used to cure disease like cholera. *Pogostemon cablin* (patchouli) is an important herb which possesses many therapeutic properties and is widely used in the

fragrance industries. In traditional medicinal practices, it is used to treat colds, headaches, fever, nausea, vomiting, diarrhea, abdominal pain, insect and snake bites. In aromatherapy, patchouli oil is used to relieve depression, stress, calm nerves, control appetite and to improve sexual interest. Till now more than 140 compounds, including terpenoids, phytosterols, flavonoids, organic acids, lignins, alkaloids, glycosides, alcohols, aldehydes have been isolated and identified from patchouli. The main phytochemical compounds are patchouli alcohol, α-patchoulene, β-patchoulene, α-bulnesene, seychellene, norpatchoulenol, pogostone, eugenol and pogostol. Pogostemon oil complexes had strong anti-fungus effect sagainst *Candida albican, Candida tropicalis*, and *Candida krusei.* (Fabienne Deguerry,2006). *Pogostemon cablin* is known to contain phytochemicals like Tilianin, stegmasterol, Retusin, ombuin, patchoulene, seychellene etc. There is high possibility that these phytochemicals play a major role in curing candidiasis. A group of bacteria belonging to genus vibrio generally cause cholera. Vibrio infection is a common bacterial disease that affects the intestines. *Vibrio cholerae* typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food. This study focuses on the identification of the phytochemical of *Pogostemon cablin* responsible to cure cholera caused by*Vivrio cholerae.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that pogostemoncablincontains Tilianin, Retusin, stigmasterol, seychellene, patchoulene, ombuin etc. It has already been established that pogostemon cablinplant belonging to lamiaceae family has potential to help controlling cholera. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of cholera.

Enzyme found in *Vibrio cholerae*

It has been reported that cholera can cause as a result of *vibrio cholerae* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Vibrio cholerae* bacteria. It has been found that protein Transcriptional activator HlyU enzyme (protein database code 4YDO) is involved in glycerolipid metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the pogostemon cablinplant were downloaded from the website. The protein database code of the Transcriptional activator HlyU enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the C DOCKER protocol of BIOVIAsoftwere under “receptor-ligand

interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-C DOCKER_ENERGY" and "-C DOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Transcriptional activator HlyU enzyme. It appears as light green color. CDOCKER is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-C DOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -C DOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -C DOCKER energy and b)small difference between -C DOCKER energy and -C DOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019, 56(2), 111-121). Table 1 shows that Transcriptional activator HlyU-tilianin interaction has the highest positive value of -C DOCKER energy (27.0095) and minimum value of the difference (10.075) between - C DOCKER interaction energy and - C DOCKER energy followed by hygrine. Thus the results indicated that tilianin, retusin, ombuincan effectively deactivate the Transcriptional activator HlyU enzyme thereby interrupting the biological cycle of *Vibrio cholerae.* Higher positive values for tilianin indicated that it was the most active ingredient against *Vibrio cholerae.* On the other hand, sey chellene steg masterol patchoulene can deactivate the enzyme to a small extent (negative -C DOCKER energy but positive -C DOCKER interactions. Thus, the key phytochemicals preventing cholera caused by Vivrio cholerae are Tilianin, Retusin and Ombuin.

CONCLUSION

It was previously known that pogostemon cablin plant has medicinal action against cholera. Cholera is caused by *Vibrio cholerae.*This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical (Tilianin, Retusin, ombuin,patchoulene,sey chellenesteg masterol), which can have a significant interaction with the vital enzyme (Transcriptional activator HlyU) of the microbe. It was found that ombuin Tilianin, Rhamnetin, ombuin and Retusin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Seychellenestegmasterol patchoulene were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of ombuin, Tilianin, Retusin and Rhamnetin provided the medicinal values to *Pogostemon cablin* against cholera caused by *Vibrio cholerae.*

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Table 1. Results of CDocking of phytochemicals with protein farnesyltransferase(receptor)

SL.NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Tilianin	14.6328	49.8024	35.1912
2	Retusin	17.2008	41.1981	23.9973
3	Ombuin	31.1651	41.028	9.8629
4	Rhamntin	31.765	41.5023	9.7373
5	Seychellene	-41.3932	23.206	64.5992
6	Patchoulene	-54.1115	24 .4009	78.5124
7	Stegmasterol	-40.3304	41.7892/	82.1196

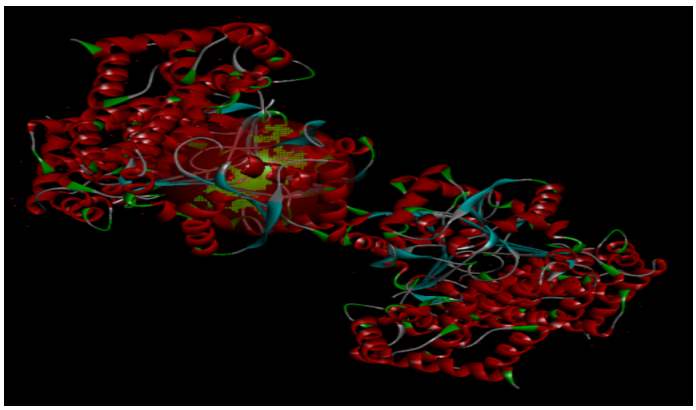

Figure 1. Active site of Transcriptional activator HlyU enzyme

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RESEARCH ARTICLE

In Silico Analysis of Phytochemicals from *Tagetes erecta (*Marigold*)* against L-Lactate Dehydrogenase of *Staphylococcus aureus* Causing Eczema

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ABSTRACT

Tagetes erecta is an ethno-medicinal plant which has very essential activity against bacteria. Multipleresearch work emphasizes the therapeutic importance of bioactive principles of *Tagetes erecta* in the treatment of eczema. This plant extract contains different phytochemicals which prevents the activity of *Staphylococcus aureus* that is causative agent of eczema. One of the important enzyme of *Staphylococcus aureus* is L-lactate dehydrogenase involved in Propanoate metabolism pathway (KEGG). The molecular docking of phytochemicals with the enzyme was studied using BIOVIA Discovery studio .The strength of the interaction was calculated based on –CDOCKER energy and –CDOCKER interaction energy. High positive values for the both the parameters indicated that out of different phytochemicals Isorhamnetin and Isoquercetin can effectively deactivate the L-lactate dehydrogenase by which life cycle of *Staphylococcus aureus* prevented.

Keywords: - Phytochemicals, BIOVIA, Discovery studio, *Tagetes erecta* ,*Staphylococcus aureus*

INTRODUCTION

Good health is the soul of satisfied life. But due to unmannered way of living individual losing greatest gift of God. In the present day, due to following unhealthy lifestyle like unhealthy diet, smoking, alcohol consumption, drug abuse, stress etc. millions of peoples are suffering from chronic diseases like obesity, diabetes, cardio vascular disease, cancer, osteoporosis, ulcer, low vision in early ages etc. According to WHO, by 2020 chronic diseases will annex the almost three quarters of the whole world. Out of them 71% death due to the cardio vascular diseases, 75% death due to diabetes will spread in the many developing countries on the world wide basis. For making a healthy society we should adopt the healthy way of living and healthy diet. We should have to avoid alcohol consumption, smoking , stress which are main factors of every diseases.

In India traditional medicinal system as well as origin of medicines is Ayurveda. The concept of Ayurveda was developed between 2500 BC to 500 BC in India. The Ayurveda empowered the system of freedom and long life. Now – a- days many synthetic drugs has side effects. For eliminating these types of problems medicines are manufactured which has fully botanical origin.According to great scholar Charaka , every plants has its own tremendous medicinal properties. Out of them enlisted here. For examples, *Moringa olifera* plants are highly effective on the diabetics, tumor, cardiac and circulatory problems. Another plant is *Rouvolfia serpentina* has effect on hypertension. Like wise, every plant has effect on the various health issues.

If plants shows medicinal properties and they can regulate the physiology of human only because of the substances called phytochemicals. These phytochemicals are secondary chemicals which has role in producing biologically active compound which protects the plants from different predators. Due to lack of side effect in medicines evolved from botanical origin many developing countries put emphasize on the research on ethno-botanicals values of plants. In India tribal groups of Nagaland used 51 species of medicinal plants which gives an idea on botanical exploration and detailed ethno-botanical studies. Expect Nagaland, tribal peoples of Madhypradesh studies on 27 medicinal plant species which is therapeutically used in diseases like acidity, diabetics, migraine, skin diseases etc. Except these some reproductive tract diseases in female and diseases like gonorrhea in male can be cure by these plants . In silent valley of Kerala also posses 102 plants which has ethno-medicinal values. These plants has effect on dermatological infections and gastrointestinal disorders. Not only these states but only in whole India ethno- medicinal values of plants are studies and Government emphasized on its research work. These research work has high economical importance in the global market.

Tagetes erecta is commonly known Marigold plant. It is called as Genda in Hindi and Gandhpushpam in Sanskrit. It belongs to familyAsteraceae.It is a herbaceous plant which is about 0.1 to 2.2m in tall.Most of the species have pinnate green leaves with fibrous roots.The disease curing activity of Marigold plant is due the presence of the phytochemicals in it. The mainphytochemiclas of *Tagetes erecta* (Marigold) areIsorhamnetin, Isoquercetin, Erythrodiol, Lupeol, Alpha-piene, Quercetin, Alpha thujene and Loliolide .There is high chance that these phytochemicals can cure eczema. During eczema the patches of skin become inflamed, itchy, red, crack and rough. Bljstors may occurs.A group of bacteria belonging to genus Staphylococcus is the causative agent of eczema belongs to family Staphylococcaceae. This study focuses on the investigation of the phytochemicals of *Tagetes erecta* responsible to cure eczema caused by *Staphylococcus aureus.*

MATERIALS AND METHODS

Software Used

Discovery studio module of BIOVIA software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Tagetes erecta* contains Isorhamnetin, Isoquercetin, Erythrodiol, Lupeol, Alpha-piene, Quercetin, Alphathujene and Loliolide etc. It has already been established that plant belonging to Celastraceae family has potential to help controlling eczema. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of eczema.

Enzyme Found in *Staphylococcus aureus*

It has been reported that eczema can cause as a result of *Staphylococcus aureus* infection.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Staphylococcus aureus* bacteria. It has been found that L-lactate dehydrogenase (protein database code 3D4P) is involved in Propanoate metabolism (Ko00640) (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Tagetes erecta* were downloaded from the website (PUBCHEM). The protein database code of the L-lactate dehydrogenase enzyme was identified from the website (RCBSPDB). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDOCKERprotocol of BIOVIA software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicator presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of L-lactate dehydrogenase. It appears as light green color. CDOCKisa molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. Thecriteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that Isorhamnetin interaction has the highest positive value of -CDOCKER energy (9.53856) and minimum value of the difference (55.87814) between - C DOCKER interaction energy and - C DOCKER energy followed by Isoquercetin. Thus the

results indicated that Isorhamnetin and Isoquercetin effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Staphylococcus aureus.* Higher positive values for Isorhamnetin indicated that it was the most active ingredient against *Staphylococcus aureus.* On the other hand Erythrodiol,Lupeol and Alpha-pieneetc can deactivate the enzyme to a small extent (negative -CDOCKER energy but positive -CDOCKER interaction energy). Oleanoic acid can't interact with the L-lactate dehydrogenase enzyme. Thus, the key phytochemicals preventing the eczema caused by *Staphylococcus aureus* are isorhamnetin and isoquercetin.

CONCLUSION

It was previously known that *Tagetes erecta* has medicinal action against eczema. Eczema is caused by *Staphylococcus aureus.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemicals Isorhamnetin , Erythrodiol, Quercetin etc can have a significant interaction with the vital enzyme L-lactate dehydrogenase of the microbe. It was found that Isorhamnetin and Erythrodiol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Lupeol, Alphapiene and Alpha thujene etc were found to be not much effective in deactivating the enzyme of the microbe. Oleanoic acid cannot deactivate the enzyme. Thus, this study could explain that the presence of Isorhamnetin and Erythrodiol provided the medicinal values to the *Tagetes erecta* against eczema by *Staphylococcus aureus.*

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Table 1: Results of Cdocking of Phytochemicals with Shikimate Dehydrogenase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Isorhamnetin	9.53856	65.4167	55.87814
2	Isoquercetin	19.6653	66.0207	46.3554
3	Erythrodiol	-95.2852	31.2048	126.49
4	Lupeol	-83.6496	41.4105	125.0601
5	Alpha piene	-2.2285	22.6932	24.9217
6	Oleanoic acid	FAILED	FAILED	FAILED

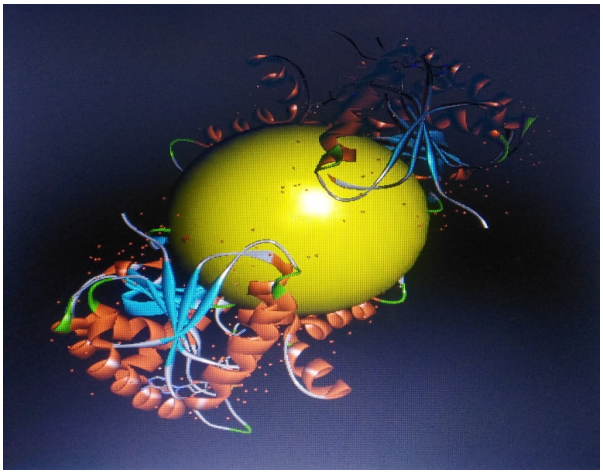

Figure 1: Active Site of L-lactate Dehydrogenase Enzyme

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RESEARCH ARTICLE

In Silico Analysis of Effects of Phytochemicals from Black Cumin against Shikimate Dehydrogenase of *Haemophilus influenzae* Causing Sore Throat

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that Black cumin plant extract is used to cure Sore throat. The plant extract contains different phytochemicals. Sore throat is caused by *Heamophillus influenzae*. One of the key enzymes involved in its biochemical pathway is Shikimate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Carvacrol and Thymoquinone can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Heamophillus influenzae.*

Keywords: - phytochemical, Biovia, Discovery studio, Black cumin, *Heamophillus influenzae*

INTRODUCTION

Life is so changed that healthy life becomes myth in today's generation.survival in olden days was hard but natural which was healthy.But in present days life becomes unhealthy due to lazyness,changing condition of work but stressful.Some good steps, little changes and care in the daily life style can prevent the diseases like diabetes, obesity, blood pressure to some extend. Plants are natural factories for the production of chemical compounds, many of which are used to promote heath and fight diseases and some of them marketed as food or herbal medicine

(Dubick,1986). Phytochemicals generally originated from the plant source are nothing but the bioactive compounds also known as secondary metabolites. These are synthesized almost all part of the plan 5 like bark, leaves, stem, root,flower,fruits,seeds etc.Also these phytochemicals present in different plant parts are used up by the local peoples for healing of certain disorders [1].Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Limited quantities of nicotine,pyrethrines and rotenine are used as pesticides.[2] Tanin are generally acts as an astringent.[3] Quinones e.g.hypericin can be used as antimicrobial agent.[4] Medicinal plants play a key role in human health care. since ancient times,people looked for cure of diseases in nature.The origin of uses of medicinal plant were not well known.The experience was the key tool for using of plant as medicine. Awareness of medicinal plants usage is the result of struggle against illness. Decrease in the efficiency of synthetic drug make the usage of natural drug topical.[5] There were 35000 to 70000 species of plants which can be used as medicinal purpose according to WHO .Medicinal plants have special properties by virtue of which drugs can be prepared from them. About 150 compounds identified in plants for the treatment of diseases know as ethno medical plants.[6]

Nigella sativa (black seed or black cumin), which belongs to the Ranunculacea family, is an annual herb with many pharmacological properties. . Black cumin is used to cure disease like sore throat (bronchodilator). [7] Black cumin is known to contain phytochemicals like 4-terpineol, Carvacrol, Limonene, Nigellidien, Thymoquinone etc.[N. K. Sharma*, D. Ahirwar, D. Jhade and S. Gupta,2009 july,Medicinal and Phamacological Potential of *Nigella sativa:* A Review,Ethnobotanical Review 13: 946-55]. There is high possibility that these phytochemicals play a major role in curing sore throat. However, there is no report identifying the specific phytochemical responsible to cure sore throat.
A group of bacteria belonging to genus *Heamophillus influenzae* generally cause sore throat. *Haemophilus influenzae* is a Gram-negative pleomorphic, anaerobic pathogenic bacterium that can be either encapsulated or unencapsulated.[8] *H. influenzae* is only found in humans and colonises the nasopharynx and throat. Transmission occurs through the spread of respiratory droplets or contact with respiratory secretions. [9] Among all the enzymes of *Heamophillus influenzae* Shikimate dehydrogenase plays an important role in the metabolic pathway like carbohydrate metabolism.[10] If the metabolic pathway blocks then the organism can't survive. So with the help of phytochemicals of Black cumin the metabolic pathway of Heamophillus influenza can be restricted. This study focuses on the identification of the phytochemical of Black cumin responsible to cure sore throat caused by *Heamophillus influenzae.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Black cumin contains 4-terpineol, Carvacrol, Limonene, Nigellidien, Thymoquinone etc. It has already been established that Black cumin plant belonging to Ranunculaceae family has potential to help controlling sore throat. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of sore throat.

Enzyme Found in *Heamophillus influenzae*

It has been reported that sore throat can cause as a result of *Heamophillus influenzae sp.* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Heamophillus influenzae sp.* bacteria. It has been found that shikimate dehydrogenase enzyme (protein database code 1P77) is involved in carbohydrate metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the Black cumin plant were downloaded from the website. The protein database code of the shikimate dehydrogenase enzyme was identified from the website (www.rcsb.org). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the shikimate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molhecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda1, Deepu Mathew2, MR Shylaja1, P Sangeetha Davis3, K Anita Cherian4, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019, 56(2), 111-121). Table 1 shows that shikimate dehydrogenase-carvacrol interaction has the highest positive value of -CDOCKER energy (19.1395) and minimum value of the difference (0.8596) between - C DOCKER interaction energy and - C DOCKER energy followed by Thymoquinone. Thus the results indicated that carvacrol and Thymoquinone can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Heamophillus influenzae*. Higher positive values for Carvacrol indicated that it was the most active ingredient against *Heamophillus influenzae sp.* On the other hand, 4-terpineol, Limonene can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Nigellidien cannot interact with shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing sore throat caused by *Heamophillus influenzae sp.* are Carvacrol and Thymoquinone.

CONCLUSION

It was previously known that black cumin plant has medicinal action against sore throat. Sore throat is caused by *Heamophillus influenzae sp.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (4-terpineol, Carvacrol, Limonene, Nigellidien, Thymoquinone), which can have a significant

interaction with the vital enzyme (shikimate dehydrogenase) of the microbe. It was found that Carvacrol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. 4-terpineol, Limonene, Nigellidien, Thymoquinone were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Carvacrol provided the medicinal values to Black cumin against sore throart caused by *Heamophillus influenzae.*

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Table 1. Results of CDocking of Phytochemicals with Shikimate dehydrogenase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	4-terpineol	-11.7948	17.8229	29.6177
2	Carvacrol	19.1395	19.9991	0.8596
3	Limonene	-21.8091	17.3439	39.153
4	Nigellidien	Failed	Failed	NA
5	Thymoquinone	2.4315	18.6375	16.206

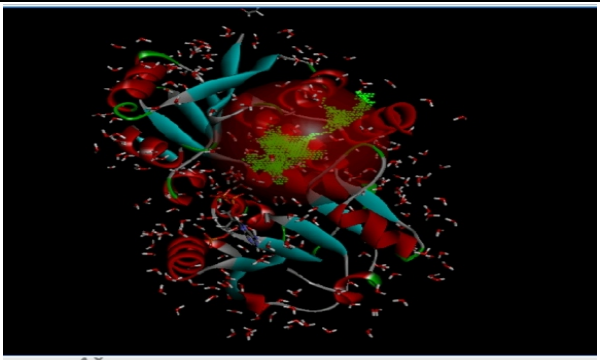

Figure 1: Active Site of Shikimate Dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Black Pepper against Malaria

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ABSTRACT

Dried *Piper nigrum* Linn .fruits, also called as black pepper is an provident valuable medicinal plant that has diverse phytochemical activities .Black pepper is one of the significant and oldest spice . It is known as the king of spice and precious for its hot and flavor [1]. It has been reported that Black pepper plant extract is used to cure malariae. The plant extract contains different phytochemicals. Malariae is caused by *Plasmodium malariae.*One of the key enzymes involved in its biochemical pathway is L-lactate dehydrogenase *Plasmodium malariae*.The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy highest valuesfor both the parameters indicated that out of different phytochemicalp- cymene can effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the life cycle of *Plasmodium malariae* .

Keywords: - Phytochemical, Biovia, Discovery studio, Black pepper, *Plasmodium malariae.*

INTRODUCTION

In time long past, life was natural, slow, difficult at times but health. Medicinal herb as a source of medication have been used throughout the globe in various cultures .Evedence shows that eariy humans relied upon natural resources including plant in order to treat various diseases. According to WHO about 80% of world's population use herbal and traditional medicines as a whole or part of the treatment. It is also started that about 25% of all modern medicines are directly or indirectly obtained from plants [2] . Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc.(Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Devi P.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Medicine uses of Black pepper include antibacterial, antifungal, antiapoptotic, antidepressant, antidiarrheal, anti–inflammatory, antimutagenic, antioxidative, antipyretic, antispasmodic, antitumor, to improve appetite and digestive power, cold, cough. dyspnea for curing from cancer fighter malariae .[3] .

Black pepper belongs to family piperaceae. Black pepper is one of the most widely used in the world .There is a distinct and undeniable earthiness to the flavour of black pepper , one that woody , piney , and sharp all at the same time . There are vast healthbnifits attributed to black pepper . For hundreds of years ,it has been a key element in a wide variety of natural remedies from mixtures used to detoxify and clear the skin to massage oils that are popular in Ayurvedic treatments Black pepper leaves extract is used to cure disease like malariae. Black pepper is known to contain phytochemicals like β-pinene, γ-pinene,piperazine,p-cymene,limonene,quercetin etc.. There is high possibility that these phytochemicals play a major role in curing malariae[4]. However, there is no report identifying the specific phytochemical responsible to cure malariae.

A group of bacteria belonging to genus *Plasmodium malariae* generally cause malariae. They are crescent and banana shape. *Plasmodium malariae* infection is a common protozoal and human pathogen disease This parasite is the most frequent and widely distributed cause of recurring malariae. *Plasmodium malariae* bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through mosquitoes.[5] This study focuses on the identification of the phytochemical of Black pepper responsible to cure malariae caused by *Plasmodium malariae.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Black pepper contains, β-pinene, γ-pinene, piperazine, p-cymene, limonene, quercetin etc. It has already been established that Black pepper plant belonging to piperaceae family has potential to help controlling malariae. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of malariae.

Enzyme Found in *Plasmodium malariae*

It has been reported that malariae can causeas a result of *Plasmodium malariae* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Plasmodium malariae* bacteria. It has been found that L-lactate dehydrogenase *Plasmodium malariae* enzyme (protein database code 2A94) is involved in L-lactaldehyde degradation and Lactate fermentation (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the Black pepper plant were downloaded from the website . The protein database code of the L-lactate dehydrogenase enzyme was identified from the website The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the -CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the L-lactate dehydrogenase enzyme. It appears as light greencolor.-CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that L-lactate dehydrogenase enzyme-p-cymene interaction has the highest positive value of -CDOCKER energy (4.64072) and minimum value of the difference (10.11498) between - C DOCKER interaction energy and - C DOCKER energy followed by piperazine. Thus the results indicated that can effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Plasmodium malariae.* Higher positive values for P-cymene and piperazine indicated that it was

the most active ingredient against *Plasmodium malariae.* On the other hand, β-pinene, γ-pinene, piperine and limonenecan deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing malariae caused by *Plasmodium malariae.*

CONCLUSION

It was previously known that Black pepper plant has medicinal action against malariae.The human being used plants as the source of medicine since ancient time. spices were part of these ancient traditional medicines .Malariae is caused by *Plasmodium malariae.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (piperine, limonene, β-pinene, γ-pinene, camphor, p-cymene , quercetin), which can have a significant interaction with the vital enzyme L-lactate dehydrogenase of the microbe.It was found thatp-cymene and piperaizinecan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. β-pinene, Alpha-pipene,piperin and limonene were found to be not much effective in deactivating the enzyme of the microbe.Thus, this study could explain that the presence of p-cymeneandpiperaizine provided the medicinal values to Black pepper against malariae caused by *Plasmodium malariae.*

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Table 1. Results of Cdocking of Phytochemicals with L-Lactate Dehydrogen (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	p-cymene	4.64072	14.7557	10.11498
2	piperaizine	4.30809	17.1484	12.84031
3	limonene	-40.7415	13.3128	54.0543
4	Beta-pinene	-78.8884	-13.2283	65.6601
5	Alpha-pipene	-111.241	-23.0981	88.1429
6	piperin	-17.7503	19.1332	36.8835

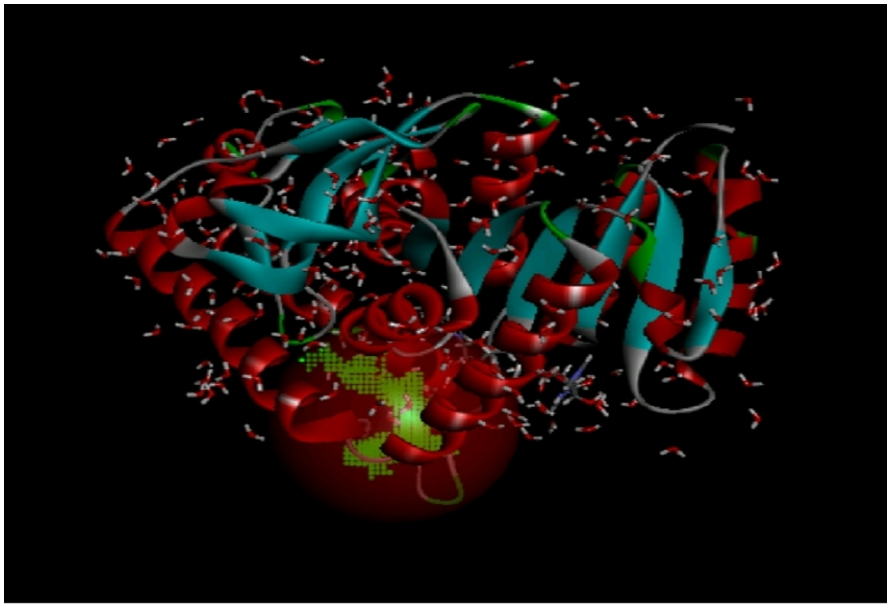

Fig.1. The active site of the L-lactate dehydrogenase enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Piper nigrum* against Peptidase of *Bordetella pertussis* Causing Cough

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ABSTRACT

The medicinal values of these plantslies in bioactive molecule or non nutritiv ecompounds known as phytochemicals that produce definite phisyological action in human body. The potential threats to plants include bacteria, viruses, fungi etc.when these plants or their parts are consumed by humans these phytochemicals fight off threats to health *Piper nigrum* is known to contain phytochemicals like Beta - pinene, alpha-pinene, lymonene, piperine, quercetin etc. There is high possibility that these phytochemicals play a major role in curing cough. Biovia software was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.It has been reported that cough can cause as a result of *Bordetella pertussis* infestation. It has been found that peptidase protein database code 3IVL is involved in protein export metabolism (KEGG) and very crucial for survival of the particular microbe.The molecular docking operation was performed to identify the phytochemical beta-pinene, alpha-pinene, piperazine, p-cymene, limonene, piperine, quercetin which can have a significant interaction with the vital enzymepeptidase of the microbe. This study could explain that the presence of p-cymene and piperazine provided the medicinal values to Piper nigrum against cough caused by *Bordetella pertussis*.

Keywords: - Phytochemical, Biovia, Discovery studio, *Piper nigrum, Bordetella pertussis.*

INTRODUCTION

Plants which have one or more parts that contains many subastances that can be used for treatment of many diseaes known as medicinal plant,Medicine derived from plants are widely famous due to their safety,healthy, easily availibilty,and low cost. The medicinal values of these plantslies in bioactive molecule or non nutritiv ecompounds known as phytochemicals, that produce definite phisyological action in human body. Some of most important

phytochemicals arealkaloids, flavonoids, terpenoids, phenolic compound, saponins and many more natural compounds act as foundation of manufacturing of many modern medicines or today prescribed medicines.(2) The primitive man started to distinuish between usefull and harm full effect of plant and collect the information. But now a days this process are reduced, y In olden era, peoples are so simple , life was very difficult, natural, slow, peoples are eating many traditional food that is good for health and peoples are so much healthy. Now, in modern days, life is very fast paced, complicated ,comfortable, eating many fast food, junk food, smoking dinking, readymade, stressful and unhealthy. Due to Changing work condition, lack of physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has causes many dangerous health diseases like blood pressure, diabetes, obesity, thyroid, arthritis etc. If we are concuss in these factors and change our lfe style and take care of ourr self we can decrease disease level.There are several or uncountable medicinal plant in the earth. These phytochemicals are derived from many parts of the plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Medicinal plants are renewable raw materials for preparation of many medicines. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. and this properties received research attetion. (Ullah N., et al.2011).Medicinal plants play a key role in human health care. Medicinal plants also help in preparation of traditional medicine and it is less cost effective.(Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in preparation of medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R.,2014). Recently WHO (World Health Organization) proved that 80% of thr people worldwide rely on herbal medicines for treatment ma ny health problem. According to WHO 21,000 plants species have the potential for being used as medicinal plants.(3)

About 25% of modern pharmaceutical drugs have botanical origins, For example, the most important phytochemicals Taxol is a natural compound of the taxoid family and comes from the pacific yew tree. It has an antitumor activity against breast cancer. The herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. Quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Treatments in medicinal plants are no or minimal side effect or safe. Medicinal plant increase the economical importance. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). *Piper nigrum* belongs to family Piperaceae. It is a perenial climbing vine, height is 10 meter, aerial root and leaves are alternatively arranged. When fruit is fully mature the diameter is about 5mm, dark red in colour, and cotains a single seed. Black paper is native to the Malabar coast of India. It was widel cultivated in the tropics of Southeast Asia. It grown in wide range of soil with Ph 5.5 to 6.5, temperature 26 to 28 C .The plant extract is used to cure disease like cough. Piper nigrum is known to contain phytochemicals like Beta -pinene, alpha-pinene, lymonene, piperine, quercetin etc. The friuts are used to produce black, white, and green pepercorn which are commonly used as spice in cooking. *Piper nigrum* also use to curearthritis, asthma, bronchits, stomach pain etc. There is high possibility that these phytochemicals play a major role in curing cough. However, there is no report identifying the specific phytochemical responsible to cure cough. The *Piper nigrum* has high anti oxidant activity, amnti-inflamatory properties, reduce blood sugar level, cholesterol, cancer fighting properties.(4)

A group of bacteria belonging to family Alcaligenaceae *Bordetella pertusis* generally cause cough. These bacteria are Gram-negative, aerobic, pathogenic, encapsulated, motileand flagellum like structure, cocobacillus. Bordetella infection is common bacterial disease that affacts the respiratory tract. These bacteria produce many toxin like pertusis toxin,adenylate cyclase toxin etc it produce no phlegm symptoms like tightness of chest and a tickle in the throat, shortness of breath, wheezing, allergy. The infection gets in to our body through nose, mouth or eyes. When

an infected person coughs or sneezes, baceria containing droplets spread in the air ,if we are close enough and can breath in these drplets ,they can enter in to nose, mouth or eye. (5) *Bordetella pertusis* typically live in mouth, nose and throat of humans. Humans become infected most frequently through contaminated air, water or food.This study focuses on the identification of the phytochemical of *Piper nigrum* responsible to cure Cough caused by Bordetella pertussis.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.when these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that piper nigrum contains beta-pinene, alpha-pinene, piperazine, p-cymene limonene, piperine, quercetin etc. It has already been established that *Piper nigrum* plant belonging to piperaceae family has potential to help controlling cough . this work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of cough.

Enzyme Found in *Bordetella pertussis*

It has been reported that cough can cause as a result of *Bordetella pertussis* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Bordetella pertusis*. It has been found that peptidase protein database code 3IVL is involved in protein export metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Piper nigrum* plant were downloaded from the website. The protein database code ofpeptidase the enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of peptidase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).

Table 1 shows that peptidase -p-cymene interaction has the highest positive value of -CDOCKER energy (20.5021) and minimum value of the difference (0.6009) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that p-cymene, piperazinecan effectively deactivate the enzyme thereby interrupting the biological cycle of *Bordetella pertussis*. Higher positive values for indicated that it was the most active ingredient against *Bordetella pertussis.* On the other hand alpha pinene, limonene can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing cough caused by *Bodetella pertussis* are p-cymene and piperazine.

CONCLUSION

It was previously known that Piper nigrum plant has medicinal action against Cough. Cough is caused by *Bordetella pertussis*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical beta-pinene, alpha-pinene, piperazine, p-cymene, limonene, piperine, quercetin which can have a significant interaction with the vital enzyme peptidase of the microbe. It was found that p- cymene and piperazine can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. alpha pinene, limonene were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of p-cymene and piperazine provided the medicinal values to Piper nigrum against cough caused by *Bordetella pertussis*.

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Table 1. Results of Cdocking of Phytochemicals with Peptidase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Beta-pinene	0.984118	21.4863	20.502182
2	Alpha- pinene	-3.37608	21.7223	25.09838
3	Piperazine	6.54383	16.2478	9.70397
4	P-cymene	20. 5021	21.103	0.6009
5	Limonene	-18.1849	21.1323	39.3172

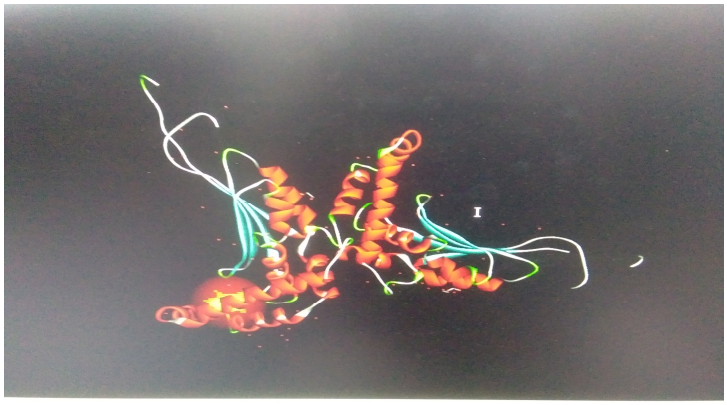

Figure 1. Active Site of Peptidase enzyme

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RESEARCH ARTICLE

In silico Analysis of Effect of Phytochemicals from Blueberry against L-Aspartate Oxidase of *Escherichia coli* Causing Urinary Tract Infection (UTI)

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ABSTRACT

Phytochemicals are non-nutritive secondary metabolites compounds obtained from plants having therapeutic activities. It has been reported that Blueberry plant extract is used to cure urinary tract infection. The plant extract contains different phytochemicals. Urinary tract infection is caused by *Escherichia coli.* One of the key enzymes involved in its biochemical pathway is L-aspartate oxidase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Caffeic acid and Luteolin can effectively deactivate the L-aspartate oxidase enzyme thereby interrupting the life cycle of *Escherichia coli.*

Keywords: - Phytochemical, Biovia, Discovery studio, Blueberry, *Escherichia coli*

INTRODUCTION

The ratio of healthy life of old ages to the healthy life of modern era gradually increases as the modernisation leads to lethal life style. Less physical activity, toxic food, mental stress are the main causes of various hazardous diseases like diabetes, hypertension, obesity etc. Some preventive caution and care should be taken to overcome the health issues of recent years.

In the humanity history health and plant have been correlated. Increased intake of fruits and vegetables is recommended in dietary guidelines worldwide because they are rich in nutrients and phytochemicals.

Phytochemicals are mainly the plant product and have a great biological activity like in therapeutic purpose. Plants derived medicinal constituents can be obtained from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). When conventional medicine dont help, people use alternative medicine almost 60% of world's population. Both in developed and developing countries alternative medicines are used for health care.[1]

Medicinal plants are the source of many drugs prescribed by the modern midicinal field. phytochemicals like curcumin, anthocyanin help in reducing the inflammation through inhibition of production of prostaglandins,the endothelial function of cardiovascular diseased patient can be improved by Lycopene present in Tomatoes, inhibition of cell proliferation and increased apoptosis can be occured by Lycopene and beta-carotene.[2]. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.
Blueberry belongs to family Ericaceae.[3] Blueberry leaves extract is used to cure disease like urinary tract infection.[4] Blueberry is known to contain phytochemicals like Solanine, Beta carotene, Caffeic-acid, Lycopene, Luteolin, Xanthone etc.[5] There is high possibility that these phytochemicals play a major role in curing urinary tract infection. However, there is no report identifying the specific phytochemical responsible to cure urinary tract infection.

A group of bacteria belonging to genus *Escherichia coli* generally cause urinary tract infection. [6] They are rod shaped Gram negative bacteria. A UTI typically starts with periurethral contamination by a uropathogen residing in the gut, followed by colonization of the urethra and subsequent migration of the pathogen to the bladder, an event that requires appendages such as flagella and pili [7]. This study focuses on the identification of the phytochemical of Blueberry responsible to cure urinary tract infection caused by *Escherichia coli.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Blueberry contains Solanine, Beta carotene, Caffeic-acid, Lycopene, Luteolin, Xanthone etc. It has already been established that Blueberry plant belonging to Ericaceae family has potential to help controlling urinary tract infection. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of urinary tract infection.

Enzyme Found in *Escherichia coli*

It has been reported that urinary tract infection can cause as a result of *Escherichia coli* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. Thesemetabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Escherichia coli* bacteria. It has been found that L-aspartate oxidase enzyme (protein database code 1KNP) is involved in Nicotinate and nicotinamide metabolism [8, 9] and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the Blueberry plant were downloaded from the website. The protein database code of the L-aspartate oxidase enzyme was identified from the website.[9] The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The“-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the L-aspartate oxidase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019, 56(2), 111-121). Table 1 shows that L-aspartate oxidase-Caffeic acid interaction has the highest positive value of -CDOCKER energy (23.6191) and minimum value of the difference (2.3731) between - C DOCKER interaction energy and - C DOCKER energy followed by Luteolin and Xanthone. Thus the results indicated that Caffeic acid can effectively deactivate the L-aspartate oxidase enzyme thereby interrupting the biological cycle of *Escherichia coli*. Higher positive values for Caffeic acid indicated that it was the most active ingredient against *Escherichia coli*. Solanine, Beta carotene, Lycopene cannot interact with L-aspartate oxidase enzyme. Thus, the key phytochemicals preventing urinary tract infection caused by *Escherichia coli* are Caffeic acid, Luteolin, Xanthone .

CONCLUSION

It was previously known that Blueberry plant has medicinal action against urinary tract infection. Urinary tract infection is caused by *Escherichia coli.This* study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Solanine, Beta carotene, Caffeic-acid, Lycopene, Luteolin, Xanthone), which can have a significant

interaction with the vital enzyme (L-aspartate oxidase) of the microbe. It was found that Caffeic acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe followed by Luteolin, Xanthone. Solanine, Beta carotene, Lycopene cannot deactivate the enzyme. Thus, this study could explain that the presence of Caffeic acid provided the medicinal values to Blueberry against urinary tract infection caused by *Escherichia coli.*

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Table 1. Results of CDocking of Phytochemicals with L-aspartate Oxidase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Solanine	Failed	Failed	NA
2	Beta carotene	Failed	Failed	NA
3	Caffeic acid	23.6191	25.9922	2.3731
4	Lycopene	Failed	Failed	NA
5	Luteolin	20.7282	23.946	3.2178
6	Xanthone	11.8661	18.7878	6.9217

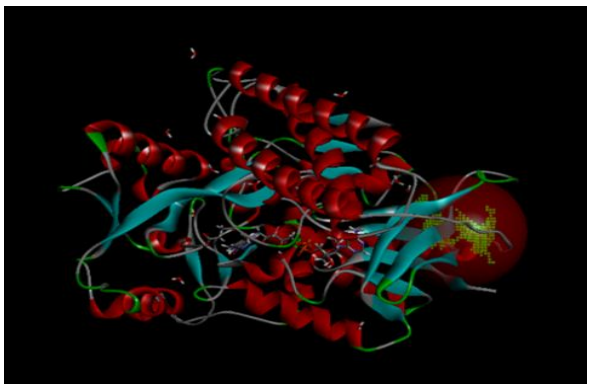

Figure 1: Active Site of L-aspartate Oxidase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Zingiber officinale* against *Haemophilus influenzae* causing Bronchitis

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ABSTRACT

Phytochemicals are non-nutritive compounds formed from plants. It has been investigated that *Zingiber officinale* plant extract is used to relieve bronchitis [1]. The plant extracts having different phytochemicals. Bronchitis is caused by *Haemophilus influenzae*. One of the key enzymes involved in its biochemical pathway is Adenine specific DNA methyltransferase enzyme. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters designated that out of different phytochemicals Gingerolcan effectively deactivate the enzyme thereby infringing the life cycle of *Haemophilus influenzae*.

Keywords: - Phytochemical, Biovia, Discovery studio, *Zingiber officinale, Haemophilus influenzae.*

INTRODUCTION

In previous days, life was natural, slow, difficult at times but healthy. Today, in present day, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, jobs settled, comfortable but pressured life and bad eating habits has given us to some dangerous diseases like blood pressure, diabetes, obesity etc. A little attention, small changes in lifestyle and care if taken, Life style can prevent related diseases from increasing.

Natural world has been a source of medicinal agents for millions of years and an effective number of modern drugs have been derived from source of natures [2]. The medicinal value of the plants lies in some chemical substances that form a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be produced from any part of plant like bark,

leaves, flowers, roots, fruits, and seeds [3]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. [4]. Medicinal plants play a key role in human health care. About 80% of the world population dependson the use of traditional medicine, which is primarily based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness means low price [5]. Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy [6]. Among different sources of natural products, plants have been a source of novel chemical substance, which gives as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the basics of many drugs prescribed today in current medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. [7]. Research requireshuge amount of medicinal plants fields, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and identification of biological activity of medicinal plants . Evidence for the beneficial effects of selected plants is generally depended on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. [8]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Zinger belongs to family zingiberaceae [9].Its extract is used to cure disease like bronchitis [1]. Zinger is known to contain phytochemicals like Gingerol, Quercetin, Panadol, Rutin, Zerumbone etc [10]. Ginger is an erect, herbaceous,perennial plant, grown for its edible rhizome .Zinger is a flowering plant whose rhizome,ginger root or ginger is widely used for spice and medicine[11].There is high possibility that these phytochemicals play a major role in curing bronchitis. However, there is no report identifying the specific phytochemical responsible to cure bronchitis.

A group of bacteria belonging to family Pasteurellaceae generally cause Bronchitis. Bronchitis disease spreads by airborne respiratory droplets ,by saliva by touching a contaminated surface ,by skin to skin contact. Symptoms of bronchitis include coughing up thickened mucus and shortness of breath. [11] This study focuses on the identification of the phytochemical of Zingiber officinale responsible to cure bronchitis caused by *Haemophilus influenzae*.This study focuses on the identification of the phytochemical of *Zingiber officinale* responsible to cure bronchitis caused by *Haemophilus influenzae.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional

medicine. Published works showed that *Zingiber officinale* contains phytochemicals like Gingerol, Quercetin, Panadol, Rutin, Zerumbone etc. It has already been established that *Zingiber officinale* plant belonging to zingiberaceae family has potential to help controlling bronchitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of bronchitis.

Enzyme Found in *Haemophilus influenzae*

It has been reported that bronchitis can cause as a result of *Haemophilus influenzae* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Haemophilus influenzae* bacteria. It has been found that Adenine specific DNA methyl transferase enzyme (protein database code 2NP7) is involved in adenine methylation of bacterial DNA (KEGG), (BRENDA) and very crucial for survival of the specific microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the Zinger plant were downloaded from the website [13]. The protein database code of the Adenine specific DNA methyltransferase enzyme was identified from the website [14]. The active site of the enzyme was identified via “receptor cavity” protocol brenda-enzymes.org found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_ INTERACTION_ ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of Adenine specific DNA methyl transferase enzyme.It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy [15].

Table 1 shows that (Gingerol) - (Adenine specific DNA methyl transferase) interaction has the highest positive value of -CDOCKER energy (35.582) and minimum value of the difference (9.6646) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that Gingerolcan effectively deactivate the enzyme thereby interrupting the biological cycle of *Haemophilus influenzae.* Higher positive values for Gingerol indicated that it was the most active ingredient against the microbe followed by Quercetin and Panadol. On the other hand, Zerumbone can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Rutin cannot interact withthe enzyme. Thus, the key phytochemical preventing bronchitis caused by *Haemophilus influenzae*is Gingerol.

CONCLUSION

It was previously known that zinger plant has medicinal action against bronchitis. The disease is caused by *Haemophilus influenzae.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Gingerol, Quercetin, Panadol, Zerumbone, Rutin) which can have ass significant interaction with the vital enzyme Adenine specific DNA methyl transferase of the microbe. It was found that Gingerol can form strong bond with the enzyme followed by Quercetin and Panadol successfully inhibiting the metabolic cycle of the microbe. Zerumbone found to be not much effective in deactivating the enzyme of the microbe. Rutin cannot deactivate the enzyme.Thus, this study could explain that the presence of Gingerol provided the medicinal values to *Zingiber officinale* against bronchitis caused by *Haemophilus influenzae.*

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Table-1: Results of CDocking of Phytochemicals with Adenine Specific DNA Methyl transferase enzyme (Receptor).

SL NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Gingerol	35.582	45.2466	9.6646
2	Quercetin	29.3415	36.6872	7.3457
3	Panadol	27.9048	31.0485	3.1437
4	Zerumbone	-47.1541	14.3037	61.4578
5	Rutin	FAILED	FAILED	FAILED

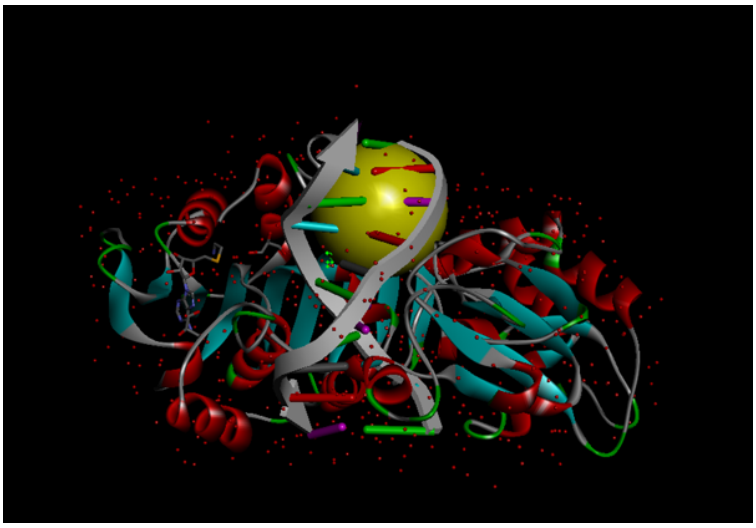

Figure 1: Active site of Adenine specific DNA methyltransferase enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Cymbopogon citratus* against Bronchitis

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Cymbopogon citratus* plant extract is used to cure Bronchitis. The plant extract contains different phytochemicals. Bronchitis is caused by *Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma Pneumoniae, Moraxella catarrhalis.* One of the key enzymes involved in its biochemical pathway is shikimate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals furfurol, Apiginin, Anthraquinones can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of the disease.

Keywords:-Phytochemical, Biovia, Discovery studio, *Foeniculum vulgare*, *Haemophilus influenzae*, *Streptococcus pneumoniae, Mycoplasma pneumoniae*, *Moraxella catarrhalis*

INTRODUCTION

Good health is the soul of satisfied life. Today, in modern times, life is fast paced, comfortable and everything is available readymade but life is stressful and unhealthy. Change in work condition, very less physical activity and jobs done by sitting. These jobs may be comfortable but are usually stressful. Irregular and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity and many other diseases which don't have or may not have any permanent solution. A little caution, very small changes in our daily lifestyle and care if taken, these lifestyle related diseases can be prevented from increasing.Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source [3].

The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds [5]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc [4]. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness [10]. Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy [12]. Among different sources of natural products, plants are a major source of novel chemical substance, which acts as starting materials for a number of different pharmaceutical products.

Medicinal plants are the foundation of many drugs which prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb silicon is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* [13]. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models [16]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Lemon Grass belongs to family poaceae. Lemon Grass extract is used to cure disease like Bronchitis [1,2,6,9] Lemon Grass is known to contain phytochemicals likeXonthones, Lutolin, Citral-quinine, Beta myrecene, apignin steroids, coumarins, furfurol, anthra quinones, phenyl propanoidsetc.[14]. There is high possibility that these phytochemicals play major role in doing away with Bronchitis. However, there is no such report identifying the specific phytochemical which is responsible to cure Bronchitis.

A group of bacteria which belong to the family pasteurellaceae are the general cause of Bronchitis. Bronchitis cause coughing, thickened mucus, shortness of breath, fatigue or malaise, runny nose, post nasal drip, chest pressure, headache, sleeping difficulty or sore throat etc. This study is focused on the identification of the phytochemical of *Cymbopogon citrates* which is responsible to cure Bronchitis which is caused by *Streptococcus Pneumoniae, Mycoplasma Pneumoniae, Moraxella catarrhalis*.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Cymbopogon citrates* contains Xonthones, Lutolin, Citral-quinine, Beta myrecene, apignin steroids, coumarins, furfurol, Anthra quinones, phenyl propanoidsetc. It has already been established that *Cymbopogon citrates* plant belonging to poaceae family has potential to help controlling Bronchitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Bronchitis.

Enzyme Found in *Haemophilus influenzae*

It has been reported that Bronchitis can cause as a result of *Streptococcus Pneumoniae, Mycoplasma Pneumoniae, Moraxela catarrhalis* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Streptococcus Pneumonia*e, *Mycoplasma Pneumonia*e, *Moraxela catarrhalis*. It has been found that shikimate dehydrogenase (protein database 1P74) is involved in Chorismate metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Cymbopogon citrates* plant were downloaded from the website [15]. The protein database code of the shikimate dehydrogenase enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site ofshikimate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019).

Table 1 shows that shikimate dehydrogenase furfurol interaction has the highest positive value of -CDOCKER energy 19.2704 and minimum value of the difference 0.2701 between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that furfurolcan effectively deactivate the enzyme thereby interrupting the biological cycle of *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae, Moraxela catarrhalis.* Higher positive values for indicated that it was the most active ingredient against *Streptococcus pneumoniae, Mycoplasma pneumoniae, Moraxela catarrhalis.*On the other hand, citral and kaempferol can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing Bronchitis caused by *Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Moraxela catarrhalis* are furfurol, Anthraquinones and Apiginin.

CONCLUSION

It was previously known that *Cymbopogon citrates* plant has medicinal action against Bronchitis. Bronchitis is caused *by Streptococcus pneumoniae, Mycoplasma pneumoniae, Moraxela catarrhalis*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical Xonthones, Lutolin, Citral-quinine, Beta myrecene, apignin steroids, coumarins, furfurol, anthra quinones, phenyl propanoids etc., which can have a significant interaction with the vital enzymeof the shikimate dehydrogenase microbe. It was found that furfurol and apiginin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Citral , kaempferol were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Furfurol and Apiginin provided the medicinal values to *Cymbopogon citrates* against Bronchitis causedby *Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma pneumoniae, Moraxela catarrhalis.*

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Table 1: Results of Cdocking of Phytochemicals with Shikimate Dehydrogenase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Furfurol	19.2704	19.0003	0.2701
2	Coumarin	15.7693	18.9411	3.1718
3	Anthraquinones	17.091	22.5775	5.4865
4	Apignin	18.5481	52.6705	34.1224
5	Citral	-11.2812	27.2834	38.5646
6	Kaempferol	-2.02663	55.952	57.97863

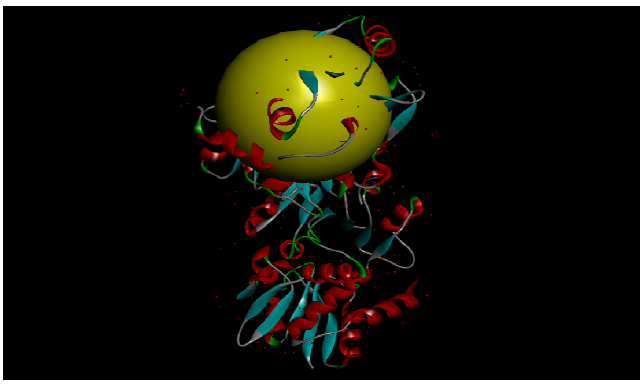

Figure 1. Active site of shikimate Dehydrogenase enzyme.

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Michelia champaca* against Shikimate Dehydrogenase of *Helicobacter pylori* Causing Chronic Gastritis

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Michelia champaca* plant extract is used to cure chronic gastritis. The plant extract contains different phytochemicals. Chronic gastritis is caused by *Helicobacter pylori.* One of the key enzymes involved in its biochemical pathway shikimate dehydrogenase enzymes.The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive values for both the parameters indicate the magnoflorine can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of chronic gastritis.

Keywords:- Phytochemical, Biovia, Discovery studio, *Champa flower.*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010).The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body,

these substances are called phytochemicals, which can be used for therapeutic purpose. Phytochemicals (From the Greek word Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Devi P.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Plumeria (/pluːˈmɛriə/) is a genus of flowering plants in the family Apocynaceae. [1] Most species are deciduous shrubs or small trees. The species variously are indigenous to Mexico, Central America and the Caribbean, and as far south as Brazil and north as Florida, but are grown as cosmopolitan ornamentals in warm regions.[2][3] Common names for plants in the genus vary widely according to region, variety, and whim, but frangipani or variations on that theme are the most common. Plumeria is also used as a common name, especially in horticultural circles.Chronic gastritis is one of the most common life-long, serious and insidious illnesses in human beings. One may estimate that more than half of the world population have this disease in some degree and extent, indicating that even many hundreds of millions of people worldwide may have chronic gastritis in a form or other.Chronic gastritis is one of the most common chronic conditions and can last for years or even a lifetime if left untreated. A wide range of different conditions and factors are known to cause or contribute to the development of chronic gastritis. Resolving mild cases of gastritis can often be through the use of medication and lifestyle changes. However, for some people with severe chronic gastritis, a cure may not be possible, and the focus of treatment will be on managing the symptoms.

The significance of chronic gastritis as a serious disease is largely underrated in clinical practice, even though the role of gastritis in the pathogenesis of ordinary peptic ulcers and gastric cancers is obvious [1, 2, 3, 4]. One may estimate that millions of premature deaths may occur annually worldwide due to cancer and ulcer as sequelae of the chronic gastritis. Chronic gastritis appears either as nonatrophic or atrophic form. They are forms and phenotypes of gastritis which represent different stages of a same life-long disease [2, 3, 5, 6, 7]. The morphological appearances of gastritis published are very similar worldwide, i.e., chronic gastritis is seemingly, with its sequelae, one and same disorder throughout the world.

Chronic gastritis has been known and studied since the early decades of the 20th century but received more attention not until 1982 after the discovery of the Helicobacter Pylori by Warren and Marshall [8]. It has become more clear that the bacterium is the cause of the gastritis in an overwhelming majority of the cases, a possible exception being a gastritis of the autoimmune origin. [3,9]. Consequently, it has become evident that the chronic gastritis can be cured with education of *H. pylori,* resulting in normalization of the gastric mucosa, at least in cases in which the gastritis is not developed to atrophic end stages [8, 9, 10,11, 12, 13].In this article, we look at the symptoms, causes, risk factors, and possible complications of chronic gastritis. We also cover when to see a doctor, diagnosis, treatment, and lifestyle and dietary changes.*H. pylori* bacterial infection is the most common cause of gastritis worldwide. Many people first become infected during childhood, but not everyone experiences symptoms.While *H. pylori* infection can cause both acute and chronic gastritis, it is not often associated with erosive gastritis.This review describes some observations and presents some subjective opinions and remarks on course and epidemiology of chronic *H. pylori* gastritis and related diseases obtained mainly from several studies in Estonia and Finland during the past 40 yrs. [14, 15, 16, 17, 18, 19, 20, 21, 22]. Over the years, most of these studies have been published in this journal. Researchers think *H. pylori* spreads through infected food, water, salvia and other bodily fluids.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Michelia champa* contain β sitosterol, gemacranolide, liriodenine, magnoflorine, micheliolide, parthenolide, etc. It has already been established that *Michelia champa* plant belonging to family magnoliaceae has potential to help controlling chronic gastritis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of chronic gastritis.

Enzyme Found in *Helicobacter pylori*

It has been reported that chronic gastritis can cause as a result of *Helicobacter pylori* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Helicobacter pylori* bacteria.It has been found that shikimate dehydrogenase has protein data base of 3PHJ. Shikimate dehydrogenase has a major role in metabolic pathway of *Helicobacter pylori* as propanol degradation it acts as a cleavage found from KEGG. Another metabolic pathway involved in helicobacter pylori is starch degradation which acts as to minimize the risks of RNA degradation.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software wasused for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Champa* plant were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the catalase enzyme was identified from the website (https:www.rcsb.org/structure/3PHJ). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_ INTERACTION_ ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the shikimate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy(OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid. Table 1 shows that shikimate dehydrogenase interaction has the highest positive value of -CDOCKER energy (3.41343) and minimum value of the difference (37.23117) between - C DOCKER interaction energy and - C DOCKER energy followed byliriodenine. Thus the results indicated that the magnoflorine and can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Helicobacter pylori.* Here liriodenine, germacranolide, parthenolide, β-sitoserol were found to be very less effective with shikimate dehydrogenase and micheliolide cannot interact with shikimate dehydrogenase. Thus the key phytochemicals preventing chronic gastritis caused by *Helicobacter pylori* is magnoflorine.

CONCLUSION

It was previously known that *Michelia champa* plant has medicinal action against chronic gastritis is caused by *Helicobacter pylori.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Germacranolide, β-sitoserol, liriodenine, magnoflorine, micheliolide, parthenolide,) which can have a significant interaction with the vital enzyme (shikimate dehydrogenase) of the microbe. It was found that magnoflorine can form better bond in comparison to other phytochemicals.Germacranolide, β-sitoserol, liriodenine, magnoflorine, micheliolide, parthenolide were found to be very less effective in deactivating the shikimate dehydrogenase enzyme. Thus, this study could explain that the presence of the phytochemicals magnoflorine provides the medicinal values to the plant *Michelia champaca* against chronic gastritis caused by *Helicobacter pylori.*

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Table 1: Results of CDocking of Phytochemicals With Shikimate Dehydrogenase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Magnoflorine	3.41343	40.6446	37.23117
2	Liriodenine	-9.67598	31.6768	41.35278
3	Germacranolide	-24.6535	26.8143	51.4678
4	Parthenolide	-31.8653	29.7605	61.6258
5	β-sitoserol	-33.4338	41.240	74.6738
6	Micheliolide	-50.3824	28.3479	78.7303

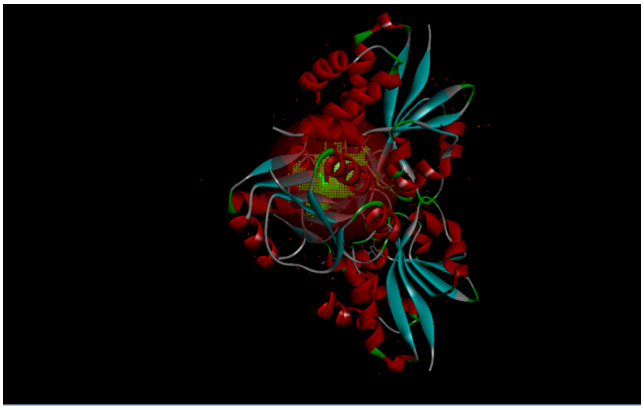

Figure 1: Active Site of Shikimate Dehydrogenase

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RESEARCH ARTICLE

In Silico Analysis of Phytochemicals from *Syzygium aromaticum* against *Porphyromonas gingivalis* Causing Oral Infections

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ABSTRACT

In past decades the modern lifestyle is leading populations to follow an unhealthy habituations pattern. With the developments of science more and more synthetic drugs from chemicals are being used which are quite effective too. But the downside of these drugs is that they contain side effects to the Individuals on the medication sometime making the matter much worse. Phytochemicals are non-nutritive compounds obtained from plants. The plant extract can contain different phytochemicals. It has been reported that *Syzygium aromaticum* plant extract can be used to cure infections in oral cavity. Oral infectionsis caused by *Porphyromonas gingivalis*. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Myricetin can effectively deactivate the microbial metabolic enzyme thereby interrupting the cycle of the infection causing microbes.

Keywords:- Phytochemical, Biovia, Discovery studio, *Syzygium aromaticum, Porphyromonas gingivalis.*

INTRODUCTION

Lifestyle includes day to day behaviors and functions of individuals in job, activities, fun and diet. In ancient days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards [1]. Moreover high production rate of synthesised dugs and easy availability leads todays populations to fall into habit of intaking of these drugs.

But with course of time it has been remarked that these drugs cause some adverse side effects on the individual which are far mor harmful and fatal in nature[2][3].

Meanwhile, Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source [4]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds [5].Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc[6]. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products while proving to be safe of side effects and cost effective [7].

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models [8]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Clove belongs to family Myrtaceae.The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. Cloves are available throughout the year due to different harvest seasons in different countries. Clove leaves extract is suspected to be used in curing disease like Oral infection. Clove is known to contain phytochemicals like Beta-caryophyllene, Compesterol 3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillin etc.[9][10] There is high possibility that these phytochemicals play a major role in curing the infections. However, there is no report identifying the specific phytochemical responsible to cure the disease.

A group of bacteria belonging to genus Porphyromonadaceae generally cause Oral infections.*Porphyromonas gingivalis* is a Gram-negative, rod-shaped, nonmotile,anaerobic, pathogenic bacterium. Mouth infections, also known as oral infections, are a group of infections that transpire around the oral cavity [11]. Mouth infections typically instigate from dental caries at the root of molars and premolars which spread to adjacent structures. In cases ofdispersal of infections to adjacent structures or in immunocompromised patients (cancer, diabetes, transplant immunosuppression), surgical drainage and systemic antibiotics may be required in addition to tooth extraction. Since bacteria that normally exist in in the oral cavity cause mouth infections, practice of proper dental hygiene can avert most cases of infection [12]. Nevertheless, Histidine kinaseis a decisive enzyme which act as a response regulater for configuration of fimbriae (fimbriation), the uniqe virulence factor of *Porphyromonas gingivalis.*[13] If the enzyme is inhibited the disease may be possibly cured by controlling the metabolic/pathogenic activities of the microbe.This study focuses on the identification of the phytochemical of *Syzygium aromaticum* responsible to cure Oral infection caused by *Porphyromonas gingivalis.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Syzygium aromaticum* contains Beta-caryophyllene, Compesterol3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillinetc. It has already been established that *Syzygium aromaticum* plant belonging to Myrtaceaefamily has potential to help controlling the infections in Oral cavity. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of the infections.

Enzyme Found in *Porphyromonas gingivalis*

It has been reported that infections in mouth can cause as a result of *Porphyromonas gingivalis* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Porphyromonas gingivalis* bacteria. It has been found that Histidine kinaseenzyme (protein database code 6BLK) is involved in fimbriation (formation of fimbriae) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Syzygium aromaticum* plant were downloaded from the website [14]. The protein database code of the Histidine kinase enzyme was identified from the website [15]. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Histidine kinase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy [16].

Table 1 show that (Myricetin)-(Histidine kinase enzyme) interaction has the highest positive value of -CDOCKER energy (29.5711) and minimum value of the difference (3.4837) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that Myricetin can effectively deactivate the Histidine kinase enzyme thereby interrupting the biological cycle of *Porphyromonas gingivalis*. Higher positive values for Myricetin indicated that it was the most active ingredient against Histidine kinase followed by Rhamnetin, Kaempferol, Gallic acid, Vanillin and Eugenol. On the other hand, Beta-caryophyllene, Stigmasterol, Compesterol 3-beta-d-glucoside, Oleanoid acidcan deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemical preventing Oral infections caused by *Porphyromonas gingivalisis* Myricetin.

CONCLUSIONS

It was previously known that *Syzygium aromaticum* plant has medicinal action against Infections in Oral cavity. This infection is caused by *Porphyromonas gingivalis*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Beta-caryophyllene, Compesterol 3-beta-d-glucoside, Eugenol, Gallic acid, Kaempferol, Myricetin, Oleanoid acid, Rhamnetin, Stigmasterol, vanillin), which can have a significant interaction with the vital enzyme Histidine kinase of the microbe. It was found that Myricetincan form strong bond with the enzyme followed by Rhamnetin, Kaempferol, Gallic acid, Vanillin and Eugenol successfully inhibiting the metabolic cycle of the microbe. While Beta-caryophyllene, Stigmasterol, Compesterol 3-beta-d-glucoside, Oleanoid acid were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Myricetin provided the medicinal values to *Syzygium aromaticum* against Oral infections caused by *Porphyromonas gingivalis*.

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Table1: Results of Cdocking of Phytochemicals with Histidine Kinase Enzyme (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Myricetin	29.5711	33.0548	3.4837
2	Rhamnetin	24.9353	29.3592	4.4239
3	Kaempferol	24.8328	29.6231	4.7903
4	Gallic acid	21.2305	19.686	-1.5445
5	vanillin	14.2487	17.4739	3.2252
6	Eugenol	6.20536	15.7619	9.55654
7	Beta-caryophyllene	-22.4782	19.7894	42.2676
8	Stigmasterol	-42.8633	39.7327	82.596
9	Compesterol 3-beta-d-glucoside	-54.8803	47.4318	102.3148
10	Oleanoid acid	-59.8683	39.5448	99.4131

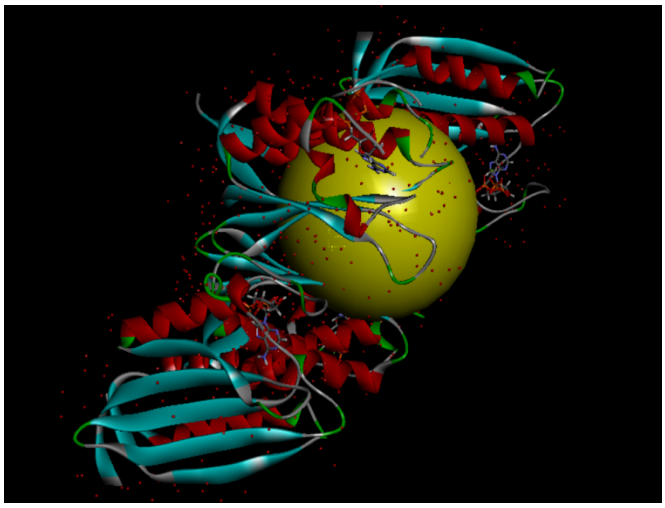

Figure 1 : Active Site f Histidine Kinase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Commiphora wightii* against Pimples

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants.Phytochemicals are substances produced mainly by plants, and these substance have biological activity. In the pharmaceutical industry, plant represents the main source to obtain various activite ingredients. They exhibit pharmacological effect application to the treatment of acne. Phytochemicals, which have protective or disease preventive properties [1] .They, are non-essential nutrient. It has been reported that *Commiphora wightii* plant extract is used to cure pimples. The plant extract contains different phytochemicals. Pimples are caused by *Propionibacteria acne sp.* One of the key enzymes involved in its biochemical pathway is D-lactate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive valuesfor both the parameters indicated that out of different phytochemicalsProgesteronecan effectively deactivate the D-lactate dehydrogenase enzyme thereby interrupting the life cycle of *Propionibacterium acnes* .

Keywords:- Phytochemical, Biovia, Discovery studio, C*ommiphora wightii,Propionibacterium acnes*.

INTRODUCTION

In ancient times, it has been proved that plants can cure disease and were the most common sources for medicines and even now in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Traditional medicine also known as indigenous or folk medicine comprises medical aspects of traditional knowledge that developd over generations within various societies before the era of modern medicine. The world health organization defines traditional medicine as the sum total of the knowledge, skills and practices based on the theories, used in the

health as well as in the prevention, diagnosis[2]. Every human being is born with a unique propotion of biologic principle ,representing the individual genetic code which take part in the formig of our mental and physical characteristics Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source. The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals, Various medicinalplants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc..Medicinal plants play a key role in human health care. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy.Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from Atropa belladonna and morphine and codeine from Papaver somniferum. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Now a day, an advice change your lifestyle is becoming common among human. There are dozens of health complaints marked as lifestyle diseases and obesity ranks top amongst those. For 3000 years, Guggulu, the gum tapped from Guggul , a Sanskrit word means protecting from disease . More than evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that defend zz anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Guggul tree is biologically known as commiphorawighti Bhandari arrived to family of burseraceeae. The gum secreted by the plant has a huge demand in international market. Guggulu is tapped by faulty extraction in order to meet the global demand .Guggulu consist of oleo -gum resin obtained as an exudate from the tapping of stem and branches of commiphorawighti it was found in arid area of India ,Gujarat ,Assam , it was produce yellowish gum . In long period guggulu has used in Ayurveda.

Commiphora wightii belongs to family apiaceae. *Commiphora wightii* leaves extract is used to cure disease like pimples. *Commiphora wightii* is known to contain phytochemicals likeD-Limonene,Z-gugglesterone,alpha-pinene,alpha-terpineol,Eugenol,Geraniol,Linalool, boroonyl acetate etc. There is high possibility that these phytochemicals play a major role in curing pimples[3]. However, there is no report identifying the specific phytochemical responsible to cure pimples.A group of bacteria belonging to genus *Propionibacterium acnes* generally cause by pimple .This study focuses on the identification of the phytochemical of *Commiphora wightii* responsible to cure pimples caused by *Propionibacterium acnes sp..*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Publishedworks showed that *Commiphora wighti* contains D-Limonene,Z-Gugglester,progesterone,alpha-terpineol,linalool,alpha-pinene,eugenol, geraniol, etc.It has already been established that *Commiphora wighti*plant belonging to Apiaceaefamily has potential to help controlling pimples. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of pimples.

Enzyme Found in *Propionibacterium acnes*

It has been reported that pimples can causeas a result of *Propionibacterium acnes sp.*infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Propionibacterium acnes sp.*bacteria. It has been found that D-Lactate dehydrogenase enzyme (protein database code2DLD) is involved Pyruvate metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Commiphora wightii* plant were downloaded from the website. The protein database code of the D-Lactate dehydrogenaseenzymewas identified from the website. The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu.Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the D-Lactate dehydrogenase enzyme. It appears as light greencolor. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda1, Deepu Mathew2, MR Shylaja1, P Sangeetha Davis3, K Anita Cherian4, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019, 56(2), 111-121).

Table 1 shows that D-Lactate dehydrogenase enzyme interaction has the highest positive value of -CDOCKER energy(35.9046) and minimum value of the difference (4.7963) between - C DOCKER interaction energy and - C

DOCKER energy. Thus the results indicated that effectively deactivate the D-Lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Propionibacterium acnes sp.*Higher positive values for progesterone indicated that it was the most active ingredient against *Propionibacterium acnes sp.*On the other hand, can deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy).Z-Gugglesterone and Bornyl acetate cannot interact with D-Lactate dehydrogenase enzyme. Thus, the key phytochemicals preventing pimples caused by *Propionibacterium acnes*.

CONCLUSION

It was previously known that *Commiphora wightii* plant has medicinal action against pimples.Pimples is caused by *Propionibacterium acnes sp.*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (D-Limonene,alpha-pinene,Z-Gugglester,progesterone,alpha-terpineol,Eugenol,geraniol,linalool), which can have a significant interaction with the vital enzyme D-lactate dehydrogenase of the microbe.It was found that Progesteroneand Eugenol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe alpha-pinene,alpha-teroineol,D-Limonene, geraniol, linalool, werefound to be not much effective in deactivating the enzyme of the microbe.borrnyl acetate and Z-Gugglesterone cannot deactivate the enzyme.Thus, this study could explain that the presence of Progesterone and Eugenol provided the medicinal values to*Commiphora wightii* against pimples caused by *Propionibacterium acnes Sp.*

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Table 1.Shows that D-Lactate dehydrogenase enzyme interaction

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Progesterone	35.9046	40.7009	4.7963
2	Eugenol	13.3917	24.1752	10.7835
3	Alpha -terpineol	-5.65296	23.1758	28.82816
4	Alpha -pipene	-9.5837	15.9241	25.5078
5	D-limonene	-18.2123	20.8225	39.0348
6	Geraniol	-18.4283	26.0639	44.4922
7	linalool	-3.09491	26.2066	29.30151

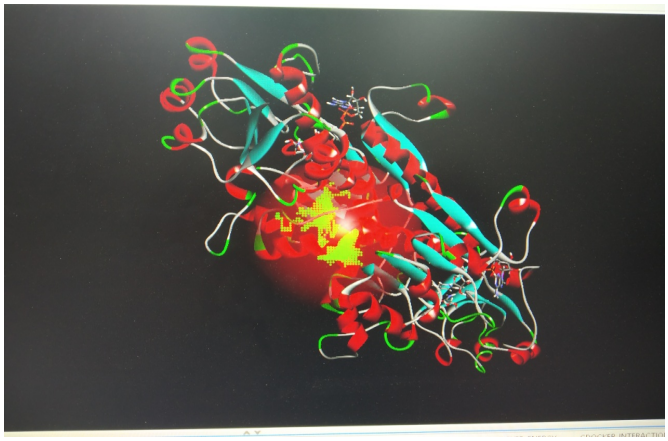

Fig. 1.Shows the active site of the D-Lactate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Celastrus paniculatus (*Malkangni*)* against Shikimate Dehydrogenase of *Haemophilus influenzae C*ausing Cough

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ABSTRACT

Celastrus paniculatus is an ethno-medicinal plant which has very essential activity against bacteria and other microbes. It has been reported that this plant extract mainly seed oil used to cure cough. Multiple research work emphasizes the therapeutic importance of bioactive principles of *Celastrus paniculatus* in the treatment of cough. This plant extract contains different phytochemicals which prevents the activity of *Haemophilus influenzae* that is causative agent of cough. One of the important enzymes of *Haemophilus influenzae* is Shikimate dehydrogenase involved in amino acid pathway. The molecular docking of phytochemicals with the enzyme was studied using BIOVIA Discovery studio.The strength of the interaction was calculated based on –CDOCKER energy and –CDOCKER interaction energy. High positive values for the both the parameters indicated that out of different phytochemicals Paraffin hydrocarbon (Benzene) and anthroquinone can effectively deactivate the shikimate dehydrogenase by which life cycle of *Haemophilus influenzae* prevented.

Keywords:- Phytochemicals, BIOVIA, Discovery studio, *Celastrus paniculatus*, *Haemophilus influenzae*

INTRODUCTION

Good health is the soul of satisfied life. But due to unmannered way of living individual losing greatest gift of God. In the present day, due to following unhealthy lifestyle like unhealthy diet, smoking, alcohol consumption, drug abuse, stress etc. millions of peoples are suffering from chronic diseases like obesity, diabetes, cardiovascular disease,

cancer, osteoporosis, ulcer, low vision in early ages etc.According to WHO, by 2020 chronic diseases will annex the almost three quarters of the whole world. Out of them 71% death due to the cardiovascular diseases, 75% death due to diabetes will spread in the many developing countries on the world wide basis. For making a healthy society we should adopt the healthy way of living and healthy diet. We should have to avoid alcohol consumption, smoking, stress which is main factors of every disease.

In India traditional medicinal system as well as origin of medicines is Ayurveda. The concept of Ayurveda was developed between 2500 BC to 500 BC in India. The Ayurveda empowered the system of freedom and long life. Now – a- days many synthetic drugs have side effects. For eliminating these types of problems medicines are manufactured which has fully botanical origin.According to great scholar Charaka, every plants has its own tremendous medicinal properties. Out of them enlisted here. For examples, *Moringa olifera* plants are highly effective on the diabetics ,tumor, cardiac and circulatory problems. Another plant is *Rouvolfia serpentina* has effect on hypertension. Likewise, every plant has effect on the various health issues.

If plants shows medicinal properties and they can regulate the physiology of human only because of the substances called phytochemicals.These phytochemicals are secondary chemicals which has role in producing biologically active compound which protects the plants from different predators.Due to lack of side effect in medicines evolved from botanical origin many developing countries put emphasize on the research on ethno-botanicals values of plants. In India tribal groups of Nagaland used 51 species of medicinal plants which gives an idea on botanical exploration and detailed ethno-botanical studies. Expect Nagaland, tribal peoples of Madhypradesh studies on 27 medicinal plant species which is therapeutically used in diseases like acidity, diabetics, migraine, skin diseases etc. .Except these some reproductive tract diseases in female and diseases like gonorrhea in male can be cure by these plants. In silent valley of Kerala also posses 102 plants which has ethno-medicinal values. This plant has effect on dermatological infections and gastrointestinal disorders. Not only these states but only in whole India ethno- medicinal values of plants are studies and Government emphasized on its research work. This research work has high economical importance in the global market.

Celastrus paniculatus is commonly known as Black oil plant. It is called as Malkangni in Hindi and Jyotishmati in Sanskrit. It is categorized as a Medhya Rasayana in Ayurveda (Nadkarni, 2002). It belongs to family Celastracea.This plant found in hilly parts of Aravalli region of Rajasthan. It is a rare and endangered medicinal plants having height up to 10 meters. The species is vulnerable in Western Ghat of south India (Rajsekharan and Ganeshan,2002). The main ethno-medicinal parts of this plant is its seed oils .These seed oils are used for memory enhancing and neuro protection (Bhanumathyet.al 2010). This plant has also anti anxiety (Rajkumar et al. 2007), anti spermatogenic (Bidwai et al. 1990),anti inflammatory activity (Ahmad et. al 1994).The oil extracted from seed of this plant highly effective for diseases like malaria, paralysis, headache , cough, and leprosy (Vaidyaratnam, 1994).It is highly active against bacteria (Patel and Trivedi , 1962).The disease curing activity of Malkangni plant is due the presence of the phytochemicals in it. The main phytochemiclas of *Celastruspaniculatus*are cardiac glycosidase (calatropin), diclofenac, anthroquinone, paraffin hydrocarbon (benzene), sucrose, and itraconajole (PUBMED) .There is high chance that these phytochemicals can cure cough.A group of bacteria belonging to genus Haemophillus is the causative agent of cough. They gram negative, cocco bacillary, facultative anaerobic bacterium belonging to Pasteurellaceae family. It can also cause respiratory infections which can affect the other organs like middle ear infection, sinusitis, meningitis, epiglottis etc. This study focuses on the investigation of the phytochemicals of *Celastrus paniculatus* responsible to cure cough caused by *Haemophilus influenzae.*

MATERIALS AND METHODS

Software Used

Discovery studio module of BIOVIA software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that *Celastrus paniculatus* contains cardiac glycoside (calatropin), diclofenac, anthroquinone, sucrose, paraffin hydrocarbon (Benzene) ,itraconajole etc. It has already been established that plant belonging to Celastraceaefamily has potential to help controlling cough. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of cough.

Enzyme found in *Haemophilus influenzae*

It has been reported that cough can cause as a result of *Haemophilus influenzae* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Haemophilus influenzae* bacteria. It has been found that shikimate dehydrogenase (protein database code1P74) is involved in biosynthesis of phenylalanine, tyrosine, and tryptophan (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software wasused for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Celastrus paniculatus* plant were downloaded from the website (PUBCHEM). The protein database code of the 1P74 enzyme was identified from the website (RCBSPDB). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDOCKERprotocol ofBIOVIA software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicatorspresented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of theshikimate dehydrogenase. It appears as light green color. CDOCKis a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MRShylaja, P Sangeetha Davis, K Anita Cherian , PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that paraffin hydrocarbon (Benzene) interaction has the highest positive value of -CDOCKER energy (12.0052) and minimum value of the difference (2.1472) between - C DOCKER interaction energy and - C DOCKER energy followed by anthroquinone.Thus the results indicated that paraffin hydrocarbon (Benzene)and anthroquinonecan effectively deactivate the shikimate dehydrogenase enzyme therebyinterrupting the biological cycle of *Haemophilus influenzae*. Higher positive values

for paraffin hydrocarbon (Benzene) indicated that it was the most active ingredient against *Haemophilus influenzae.* On the other hand cardiac glycosides (calatropin), dicofenac, sucrose, can deactivate the enzyme to a small extent (negative -CDOCKER energy but positive -CDOCKER interaction energy). Itraconajole cannot interact with the shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing the cough caused by *Haemophilus influenzae* are paraffin hydrocarbon (Benzene) and anthroquinone.

CONCLUSION

It was previously known that *Celastrus paniculatus*plant has medicinal action against cough. Cough is caused by *Haemophilus influenzae.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of BIOVIAsoftware, molecular docking operation was performed to identify the phytochemical cardiac glycosides (calatropin), diclofenac, anthroquinone, paraffin hydrocarbon (Benzene), sucrose, itraconajole) which can have a significant interaction with the vital enzyme shikimate dehydrogenase of the microbe. It was found that paraffin hydrocarbon (Benzene) and anthroquinone can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.Diclofenac, calatropin, sucrose were found to be not much effective in deactivating the enzyme of the microbe. Itraconajole cannot deactivate the enzyme. Thus, this study could explain that the presence of paraffin hydrocarbon (Benzene) and anthroquinone provided the medicinal values to the *Celastrus paniculatus* againstcough caused by *Haemophilus influenzae.*

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Table 1: Results of Cdocking of Phytochemicals With Shikimate Dehydrogenase (Receptor)

SL. NO.	LIGAND	-C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTION ENERGY AND -C DOCKER ENERGY
1	Paraffin hydrocarbon(Benzene)	12.0052	14.1524	2.1472
2	Anthroquinone	17.1348	22.7669	5.6321
3	Diclofenac	18.9082	25.9093	7.0011
4	Calatropin	-82.0636	40.8550	122.9186
5	Sucrose	-0.826655	50.846700	51.673355
6	Itraconajole	FAILED	FAILED	NA

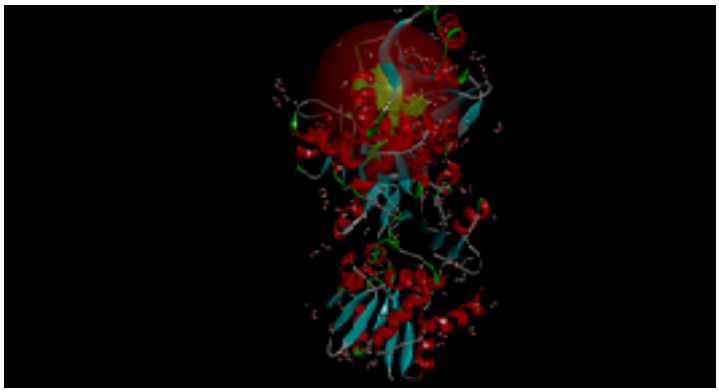

Figure 1: Active Site of Shikimate Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Cuminum cyminum* against IMP Dehydrogenase of *Campylobacter jejuni*

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Cuminum cyminum* plant extract is used to cure diarrhoea. The plant extract contains different phytochemicals. Diarrhoea is caused by *Campylobacter jejuni.* One of the key enzymes involved in its biochemical pathway is IMP dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals cuminaldehyde and coumarincan effectively deactivate the IMP dehydrogenase enzyme thereby interrupting the life cycle of *Campylobacter jejuni*.

Keywords:- Phytochemical, Biovia, Discovery studio, *Cuminum cyminum,Campylobacter jejuni*

INTRODUCTION

Diarrheal diseases are one of the major causes of mortality and morbidity worldwide, especially during the first 5 years of life for individuals subjected to malnutrition[1,2].Globally, it is estimated that there are 2.5 billion episodes and 1.5 million deaths annually in children under five years[3].Diarrheal diseases are major causes of malnutrition, delayed physical development, and early childhood mortality in developing countries and poor communities, and the major cause of death in children with diarrhoea is loss of water and essential minerals[4].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source [5].Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses. Approximately 50% of all the drugs in clinical use in the

world today related to the natural products and their derivatives. The medicinal value of the plants lies in some chemical substances that produce definite physiological actions on the human body; these substances are called photochemical, which can be used for therapeutic purpose. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety [6].Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds[7].Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc.[8].Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products. Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae. An extract of Cumin seed is used to cure disease like diarrhoea. Traditional uses of cumin include anti-inflammatory, diuretic, carminative, and antispasmodic. It has also been used as an aid for treating dyspepsia, jaundice, diarrhoea, flatulence, and indigestion. Although cumin is thought to have uses in traditional medicine, there is no high-quality evidence that it is safe or effective as a therapeutic agent [9]. Cumin is known to contain phytochemicals like 2-ethoxy-3-isopropylpyrazine, anthraquinone, coumarin, cuminaldehyde, 2-methox-3-methylpyrazine, pyrazyne [10]. There is high possibility that these phytochemicals play a major role in curing diarrhoea. However, there is no report identifying the specific phytochemicals responsible to cure diarrhoea.*Campylobacter* (meaning “curved bacteria”) is a genus of Gram negative bacteria [11]. Campylobacter is a helical-shaped, non-spore-forming, microphilic, nonfermenting bacterium forming motile rods with a single polar flagellum, which are also oxidase-positive and grow optimally at 37-420C[12,13]. When exposed to atmospheric oxygen, *C. jejuni* is able to change into a coccal form [14].Another source of infection is contact with infected animals, which often carry *Campylobacter* asymptomatically [15].*C.jenuni* is one of the most common causes of food poisoning. Infection with *C.jenuni* usually results in enteritis, which is characterised by abdominal pain, diarrhoea, fever and malaise.*C. jejuni* is also commonly found in animal feces.

Diarrhoea is characterized by loose, watery stools or a frequent need to have a bowl movement [16]. Diarrhoea can be defined by increased stool frequency, liquidity, or volume. The most common cause is an infection of the intestines due to a virus, bacteria, or parasite- a condition also known as gastroenteritis [16].These infections are often acquired from food or water that has been contaminated by feces or directly from another person who is infected [16] There are many different symptoms of diarrhoea like nausea, abdominal pain, cramping, bloating, fever, dehydration, a large volume of stools. Various preventive techniques were reported in the literatures including hygiene and sanitation, diet, medications, and supplements which are generally classified as health care, breastfeeding, immunization, supplemental zinc, and probiotics [17]. Treatment and prevention of diarrhea can be done at home by primary caregivers, and their role is vital in health promotion, disease prevention, and patient care [4].This study focuses on the identification of the phytochemical of *Cuminumcyminum* responsible to cure diarrhoea caused by *Campylobacter jejuni.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (DassaultSystems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that *Cuminum cyminum* contains 2-ethoxy-3-isopropylpyrazine,2-methoxy-3methylpyrazine,Anthraquinone,Coumarin,cuminaldehyde, pyrazyne. It has already been established that *Cuminum cyminum* plant belonging to Apiaceae family has potential to help controlling diarrhoea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of diarrhoea.

Enzyme found in *Campylobacter jejuni*

It has been reported that diarrhoea can cause as a result of *Campylobacter jejuni* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Campylobacter jejuni* bacteria. It has been found that IMP dehydrogenase (protein database code 5UQF) is involved in purine metabolism (BRENDA) [18] and is very essential for survival of the particular microbe because purine metabolism maintains cellular pools of adenylate and guanylate via synthesis and degradation of purine nucleotides.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Cuminum cyminum* plant were downloaded from the website [19]. The protein database code of the IMP dehydrogenase enzyme was identified from the website [20]. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and phytochemical was treated as the ligand. The “-CDOCKER_ENERGY and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemicals responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the IMP dehydrogenase enzyme. It appears as light greencolor. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. Thecriteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda1, Deepu Mathew2, MR Shylaja1, P Sangeetha Davis3, K Anita Cherian4, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019, 56(2), 111-121) [21].

Table 1 show that IMP dehydrogenase-cuminaldehyde interaction has the highest positive value of -CDOCKER energy (27.3498) and minimum value of the difference (2.4731) between - C DOCKER interaction energy and - C

DOCKER energy followed by coumarin. Thus the results indicated that cuminaldehyde and coumarincan effectively deactivate the IMP dehydrogenase enzyme thereby interrupting the biological cycle of *Campylobacter jejuni*. Higher positive values for cuminaldehyde indicated that it was the most active ingredient against *Campylobacter jejuni*. On the other hand, like 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-methylpyrazine, anthraquinone, pyrazyne can deactivate the enzyme to a small extent. Thus, the key phytochemicals preventing diarrhoea caused by *Campylobacter jejuni* are cuminaldehyde and coumarin.

CONCLUSION

It was previously known that *Cuminum cyminum* plant has medicinal action against diarrhoea. Diarrhoea is caused by *Campylobacter jejuni*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-methylpyrazine, anthraquinone, coumarin, cuminaldehyde, pyrazyne) which can have a significant interaction with the vital enzyme (IMP dehydrogenase) of the microbe.It was found that cuminaldehyde and coumarin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-methylpyrazine were found to be not much effective in deactivating the enzyme of the microbe.Thus, this study couldexplain that the presence of cuminaldehyde and coumarin provided the medicinal values to *Cuminum cyminum* against diarrhoea caused by *Campylobacter jejuni.*

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Table 1. Results of Cdocking of Phytochemicals with IMP Dehydrogenase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	2-ethoxy-3-isopropylpyrazine	22.2337	34.0799	11.8462
2	2-methoxy-3-methylpyrazine	12.5629	23.4941	10.9312
3	Anthraquinone	22.0974	28.0152	5.9178
4	Coumarin	23.5627	26.8815	3.3188
5	Cuminaldehyde	27.3498	29.8229	2.4731
6	Pyrazyne	7.52745	16.7733	9.24585

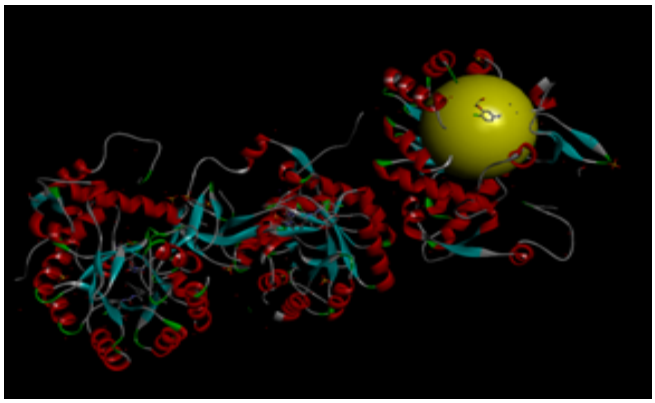

Figure 1. Active Site of IMP Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Cuminum cyminum* against Boils

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ABSTRACT

Phytochemicals are non-nutritive compounds produced from plants. It has been observed that *Cuminum cyminum* plant extract is used to prevent boils [1].The plant extract contains different phytochemicals. *Staphylococcus aureus* species cause boils. One of the key enzymes involved in its biochemical pathway is Glucose 1-dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters showed that out of different phytochemicals Cuminaldehyde can effectively deactivate the enzyme thereby injecting the life cycle of *Staphylococcus aureus*.

Keywords:- Phytochemical, Biovia, Discovery studio, *Cuminum cyminum,Staphylococcus aureus.*

INTRODUCTION

In ancient era, life was natural, slow, difficult at times but healthy. Today, in modern age, life is fast paced, comfortable, readymade, stressful and unhealthy. Work condition is changed, less physical activity, settlement of jobs, enjoyable but stressed life and bad eating habits has exposed us to some dangerous diseases like blood pressure, diabetes, obesity etc. A little attention, small changes in lifestyle and care if taken, Lifestyle can prevent related diseases from increasing.Natural world has been a source of medicinal agents for thousands years old and a grand number of modern drugs have been produced from natural source [2].The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be obtained from any part of plant like bark, leaves, flowers, roots, fruits, and seeds[3].Various medicinal plants and their

phytoextracts have shown numerous medicinal values like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc[4].Medicinal plants play animportant role in human health care. About 80% of the world population depends on the use of traditional medicine, which is basically based on plants; the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness [5]. Many of the medicinal plants are used as spices and food items. They also played a great role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy [6].Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceuticasl products.

Medicinal plants are the foundation of many drugs prescribed today in current medicinal system. About 25% of modern medicinal drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from Atropa belladonna and morphine and codeine from *Papaver somniferum* [7]. Research requires in huge amounts of medicinal plants , but are balanced by the benefits of potential health and the large size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is needed. Newly emerging scientific techniques and accesses have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models [8].Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Cumin belongs to family apiaceae[9].Extract is used to cure disease like boils [1].Cumin is known to contain Phytochemicals like 2-ethoxy-3-isopropylpyraine, 2-methoxy-3-methylpyrazine, Anthraquinone, Coumarin, Cuminaldehyde, Pyrazyne, etc.[10].Cumin is annual herbaceous plant.It consists dried seed .It has slender,glabrous ,branched stem that is 20-30 cm and has diameter of 3-5 cm .It consists dissected leaves and white or rose-coloured flowers[11].There is high possibility that these phytochemicals play a major role in curing boils . However, there is no report identifying the specific phytochemical responsible to cure boils.

A group of bacteria belonging to family *Staphylococcaceae* generally cause boils.Boils form under the skin when bacteria infect and inflame one or more hair follicles .Boils start as red, tender lumps.These fill with pus andgrows, then rupture and then drain. On the other hand, glucose 1-dehydrogenase is an enzyme that plays akey role in glycogen metabolism pathway of the microbe. By targeting this particular enzyme, the disease can be controlled by inhibiting the enzyme successfully stoping the microbial metabolic pathway.This study focuses on the identification of the phytochemical of *Cuminum cyminum* responsible to cure boils caused by *Staphylococcus aureus*.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Cuminum cyminum* contains 2-ethoxy-3-isopropylpyraine, 2-methoxy-3-methylpyrazine, Anthraquinone, Coumarin, Cuminaldehyde, Pyrazyne etc. It has already been established that

cuminplant belonging to apiaceaefamily has potential to help controlling the boils disease. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of *Staphylococcus aureus*.

Enzyme found in *Staphylococcus aureus*

It has been reported that boils can cause as a result of *Staphylococcus* species infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Staphylococcus aureus*.It has been found that glucose 1-dehydrogenase (protein database code 1GCO) is involved in glycogen metabolism (KEGG), (BRENDA) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the cumin plant were downloaded from the website[12].The protein database code of glucose 1-dehydrogenase the enzyme was identified from the website[13].The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftware under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of glucose 1-dehydrogenaseenzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy[14].

Table 1 shows that (Cuminaldehyde)- (glucose 1-dehydrogenase) interaction has the highest positive value of -CDOCKER energy (18.2806) and minimum value of the difference (2.2352) between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that Cuminaldehydecan effectively deactivate the enzyme thereby interrupting the biological cycle of *Staphylococcus aureus*.Higher positive values for indicated that it was the most active ingredient against glucose 1-dehydrogenase.On the other hand, almost each phytochemical shows a positive interaction differing only in the values which indicates the strength of the bond between the receptor and the ligand. Thus, the key phytochemicals preventing boils caused by *Staphylococcus aureus* is Cuminaldehyde.

CONCLUSION

It was previously known that *Cuminum cyminum* plant has medicinal action against boils.Boils is caused by *Staphylococcus* sp. This study was carried out to provide the theoretical basis of this observation. Using Discovery

studio module of Biovia software, molecular docking operation was performed to identify the phytochemical Cuminaldehyde, which can have a significant interaction with the vital enzymeglucose 1-dehydrogenase of the microbe. It was found that Cuminaldehyde can form strong bond with the enzyme followed by Anthraquinone, Coumrin,2-ethoxy-3-isopropylyrazine, 2-methoxy-3-methylpyrazine, Pyrazine respectively, which successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of Cuminaldehyde provided the medicinal values to *Cuminum cyminum* against boilscaused by *Staphylococcus aureus*.

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Table 1: Results of Cdocking of Phytochemicals With (Glucose 1-Dehydrogenase) (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Cuminaldehyde	18.2806	20.5158	2.2352
2	Anthraquinone	15.5861	20.9724	5.3863
3	Coumrin	14.897	18.0072	3.1102
4	2-ethoxy-3-isopropylyrazine	13.5619	23.4052	22.04901
5	2-methoxy-3-methylpyrazine	6.06357	16.4302	10.36663
6	Pyrazine	2.492	11.77085	9.2865

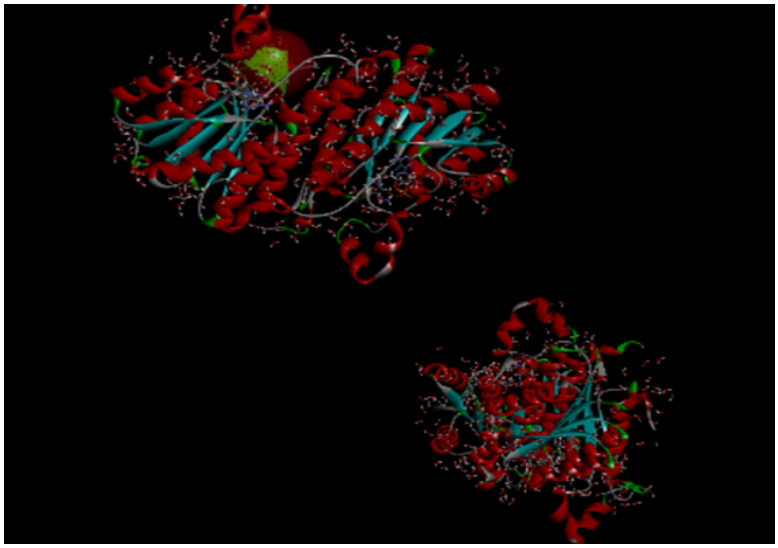

Figure 1: Active Site of Glucose 1-Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Curcuma aromatica* against Histidine kinase of *Bordetella pertussis* Causing Cough

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ABSTRACT

Curcuma aromatica, belonging to the family Zingiberaceae, is an economically important genus havingboth medicinal and food values.The plant extract contains different phytochemicals. Phytochemicals are non-nutritive compounds produced from plants. It has been reported thatCurcumaaromaticaplant extract is used to cure cough. Coughis caused byBordetella pertussis, *Bordetella parapertusis*.One of the key enzymes involved in itsbiochemical pathway is Histidine kinase. The molecular docking of the phytochemicals withthe enzyme was studied using Biovia Discovery Studio. Its rhizomes are substituted for wildturmeric and used externally to treat bruises, sprains, skin eruptions, cough, infections and to improve complexion. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interactionenergy. High positive values for both the parameters indicated that out of different phytochemicals beta pinene and Curcumin can effectively deactivate the enzyme thereby interrupting the life cycle of Bordetella pertussis. The plants have been shown to contain bioactive molecules that possess pharmacological properties like anti-inflammatory, antimicrobial, hypocholestraemic, antirheumatic, antiviral, antifibrotic, antivenomous, antihepatotoxic, antidiabetic, antinociceptive, anticancerous, and gastroprotectiveproperties.Agar diffusion method was adopted for determining the antibacterial activity of the extract.

Keywords:- Phytochemicals, BIOVIA, Discovery studio, *Curcuma aromatica,Bordetella pertussis.*

INTRODUCTION

Life is much harder in olden days, but it would have been more peaceful in olden days. Today Modern times life is different from old on all sides; Comfortable, Physical conditions,ready made and unhealthy.Changing work condition, less physical activity, sedentary jobs,comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. The change into modern life is positive life andshould be continued in control.Cautions and awareness prevent these lifestyle related diseases from increasing.

A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyles related diseases from increasing. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. The extraction and characterization of important bioactive compounds with vital medicinal properties may provide opportunities related to food and pharmaceutical applications.Plants belonging to the genus Curcuma are gaining importance globally as one of the significant ingredients in food and traditional medicines.Traditional medicinal uses, they have been used for the treatment of enlarged liver, spleen, stomach ulcer, diabetes, cough, hepatic disorders, chest pain, skin diseases, boils, blood purifier, and rheumatism.Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). It works as an antioxidant anti-inflammatory,antimicrobial, supports joint health, detoxifythe body, protect the cardiovascular system, promotes cell growth, antidepressants, balance stress hormone, anticancer.Man, since time immemorial has been using plants or natural products as medicine to promote and maintain goodhealth. Many of the medicinal plants are used as spices and food items. Plants play an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Herbal medicine is the oldest form of medical treatment. People used medicinal plants for different therapeutic purposes from ancient time. Since ancient time, also people have been exploring the nature, particularly plants in search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases. Herbal extracts used as medicine are now presently being used as a replacement for synthetic drugs.About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug Taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*.Plants play the significant role in remedy and a large number of drugs which are used the derivatives from plants. Evidence for the beneficial effects of selected plants is demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. The antimicrobial activities of the plant extract were determined by the disc-diffusion method. The tests were performed on Muller- Hinton agar for all microbes except *Streptococcus sp*. Antimicrobial activity is used in ayurveda and traditional medicinal system for the treatment of manifestations caused by microorganisms.

Curcuma aromatic belongs to family Zingiberzaceae. *Curcuma aromatica* leaves extract is used to cure disease like Cough.*Curcuma aromatica* is known to contain phytochemicals likebeta curcumene, d-camphor, alpha turmerone, beta turmerone, dcamphene, P-methoxycinolic acid, Germacrene, curzerene, germacrone, alpha pinene, beta pinene, borneol, alpha terpeniol, myrcene, terpinolene, gama terpinene, alpha copaene, alpha borgamotene,cuminic

aldehyde, betabisabolene, Cuminyl alcohol, xanthorrhizol, curcuphenol, beta elemene, zingiberene, linalool, beta farnesene, 8-cinoneole, curzerenone, curcumin, camphene, limo nine, beta thujone etc.

A group of *Bordetella pertuss* is belonging to the family Alcaligenaceae generally cause cough. Cough is a common bacterial disease that affects the breath.There is high possibility that these phytochemicals play a major role in curing cough. However, there is no report identifying the specific phytochemical responsible to cure Cough. The oil had strong detrimental effect on the viable count of the tested bacteria. The results obtained from this study may contribute to the development of new antimicrobial agents with potential applications in food industries as natural preservatives. This study focuses on the identification of the phytochemical of *Curcuma aromatica* in responsible to cure cough caused by *Bordetella parapertussis*.

MATERIALS AND METHODS

Software Used

Discovery studio module of BIOVIA software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecularinteraction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Curcuma aromatica* contains Phloretin-3-5-Di-C-glucoside, Cornstarch,3-Bromo-2Pentanol,Rosmarinicacid,(+)Catechin,1-Hexanol, liquiritigenin, Linalool-oxide-A, tanicacid, cortison, Geraniol, Sucrose, cholesterol, Isocoumarin, Pyrazine, Harmalol, Glycerrehetinic acid, glycyrrhizin, Terpinen-4-ol, alpha-Terpineol, Pectinetc. It has already been established that *Curcuma aromatica* plant belonging to Zingiberzaceae family has potential to help controlling cough.This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of cough.

Enzyme found in *Bordetella pertussis*

It has been reported that cough can cause as a result of *Bordetella pertussis* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in Bordetella pertussis. It has been found that Histidine kinase (3IVC) is involved in two component systems of pathway (bpe02020) (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the Curcuma aromaticaplant were downloaded from the website (PUBCHEM). The protein database code ofhistidine kinase. The enzyme was identified from the website (RCBSPDB). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of BIOVIAsoftware under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the

quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of Histidine kinase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods. -CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019, 56(2), 111-121) .

Table 1. shows that 21.9565- 1.47626 interaction has the highest positive value of -CDOCKERenergy 20.48024. and minimum value of the difference 7.3111between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that 20.48024 can effectively deactivate the enzyme thereby interrupting the biological cycle of *Bordetella pertussis*. Higher positive values for indicated that it was the most active ingredient against Histidine kinase of *Bordetella pertussis*. On the other hand, Histidine kinase can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Curcurene, camphene, Beta turmerone, Germacrene interact with the enzyme very loosely. Thus, the key phytochemicals preventing coughcaused by Bordetella parapertussis are Curcumin, curcumin aldehyde and beta pinene.

CONCLUSION

Curcuma aromatica is one of the most useful plants with highly potent pharmacological activities.It was previously known that *Curcuma aromatica* plant has medicinal action against cough. Cough is caused by *Bordetella pertussis*.Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical beta curcumene,d-camphor, alpha turmerone, beta turmerone, dcamphene, P-methoxycinolic acid, Germacrene, curzerene, germacrone, alpha pinene, beta pinene, borneol, alpha terpeniol, myrcene, terpinolene, gama terpinene, alpha copaene, alpha borgamotene, cuminic aldehyde, betabisabolene, Cuminyl alcohol, xanthorrhizol, curcuphenol, beta elemene, zingiberene, linalool , beta farnesene, 8-cinoneole, curzerenone,curcumin, camphene, limonine,beta thujone etc.which can have a significant interaction with the vital enzyme Histidine kinase of the microbe. It was found that Beta pinene curcumin, curcumin aldehyde can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Beta turmerone, camphene Curcurene, and Germacrene were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presencebeta pinene and curcumin provided the medicinal values to *Curcuma aromatica* againstcoughcaused byBordetella parapertussis. Researches on nutritional values along with pharmacological studies of new uninvestigated compounds are desirable and will provide immense opportunities for the development of new plant-based food and pharmaceutical products. Phytochemical screening provides knowledge of the chemical constitutes of plants not only therapeutic agents but also for information in discovering new sources of other economic materials.

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Table 1: Results of C-Docking of Phytochemicals with Histidine Kinase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	beta turmerone	-6.12909	31.4028	37.53189
2	d camphene	-43.5474	20.8864	64.4338
3	Germacrene	-16.4185	25.594	42.4057
4	beta pinene	1.47626	21.9565	20.48024
5	cuminic aldehyde	20.5452	21.9837	1.4385
6	curcumin	37.7373	45.0484	7.3111
7	camphene	-42.7199	31.4028	64.4381
8	Curcurene	-4.86897	25.594	30.46297

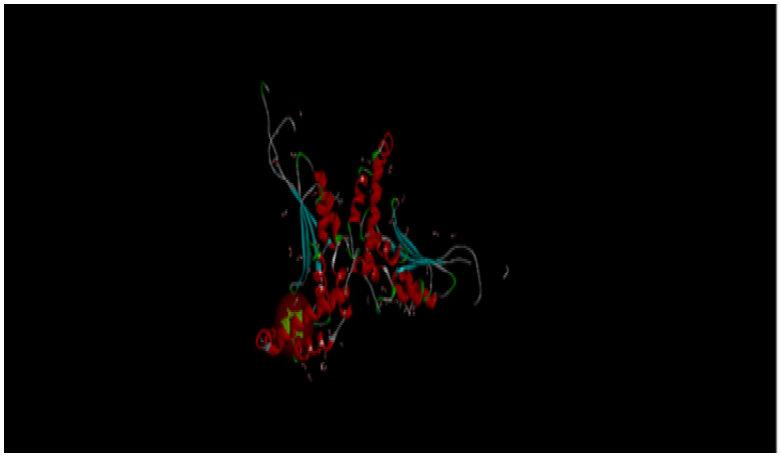

Figure 1: Active Site of Histidine Kinase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Curcuma aromatic* against Acetoacetyl-CoA Reductase of *Streptococcus equi* causing Skin Disease

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Curcuma aromatica* extract is used to cure skin disease. The plant extract contains different phytochemicals.Skin disease is caused by *Streptococcus equi.* One of the key enzymes involved in its biochemical pathway is Acetoacetyl-CoA. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive values for both the parameters indicate Curcumin and Cuminyl alcohol can effectively deactivate Acetoacetyl-CoA reductase enzyme thereby interrupting the life cycle of *Streptococcus equi*.

Keywords:- Phytochemical, Biovia, Discovery studio, *Streptococcus equi, Curcuma aromatica.*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010).The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals ,which can be used for therapeutic purpose.Phytochemicals (From the Greek word Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy (DeviP.R.,2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Curcuma aromatica is a small plant about 40cm (16in) in height belongs to family Zingiberaceae.Most of the species are perennial foliage. It dies in late autumn and rhizomes remain dominant in winter.The inflorescence appears in early spring from the base of rhizome. During summer monsoon and the immediately following weeks, the plant grows fast and vigorously. The stalk grows about 20-30cm tall and is crowned with enlarged colour bracts with pink tips.Leaves often appear even after the flowers. This species is found in the South Asian region, predominantly in east Himalayas and in the worm forests of the Western Ghats (India). *Curcuma aromatica* is known to contain phytochemiclas like Curcumin, Cuminyl alcohol, Cuminic aldehyde, Camphene, Borneol, Beta tumerone, Beta curcumene and Alpha tumerone etc. There is high possibility that these phytochemiclas play a major role in curing skin disease.How ever there is report identifying the specific phytochemiclas responsible to cure skin disease. A group of Streptococcus equi belongs to family Streptococcaceae generally cause skin disease. During skin disease the swollen of lymph nodes occur and it is very painful. This study focuses on the identification of phytochemiclas of *Curcuma aromatica* responsible to cure skin disease caused by *Streptococcus equi*.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that Curcumin,Cuminyl alcohol, Cuminic aldehyde, Camphene, Borneol, Beta tumerone, Beta pinene, Beta curcumene and Alpha tumeroneetc. It has already been established that *Curcuma aromatica* belonging to family Zingiberaceae has potential to help controlling skin disease. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of skin disease.

Enzyme found in *Streptococcus equi*

It has been reported that skin disease can cause as a result of *Streptococcus equi.*infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Streptococcus equi* bacteria.It has

been found that Acetoacetyl-CoA reductase (3Gk3) is involved in Tryptophan metabolism, Propanoate metabolism,Butanate metabolism and Synthesis and degradation of ketone bodies (KEGG) and very crucial for survival of the particular Microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Curcuma aromatic* were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the Acetoacetyl-CoA reductase was identified from the website (https:www.rcsb.org/structure/3Gk3). The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the Acetoacetyl-CoA reductase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy(OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid.Table 1 shows that Acetoacetyl-coA reductase interaction has the highest positive value of -CDOCKER energy(43.9041) and minimum value of the difference (13.5159) between - C DOCKER interaction energy and - C DOCKER energy followed Cuminyl alcohol. Thus the results indicated that the magnoflorine and can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Streptococcus equi.* Here Camphene, Borneol and Beta-pinene etc. found to be very less effective with Acetoacetyl-coA reductase and Alpha tumerone cannot interact with Acetoacetyl-CoA reductase.Thus the key phytochemicals preventing skin disease caused by *Streptococcus equi* is Curcumin.

CONCLUSION

It was previously known that *Curcuma aromatica* has medicinal action against skin disease caused by *Streptococcus equi.*This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemicals (Curcumin, Cuminylalcohol,Cuminic aldehyde, Camphene,Borneol and Beta-pineneetc) which can have a significant interaction with the vital enzyme (Acetoacetyl-CoA reductase) of the microbe. It was found that Curcumin and Cuminic aldehyde can form the strong bond with the enzyme and successfully inhibit the metabolic cycle of microbe. Camphene, Beta-pinene and Borneoletc were found to be not much effective in deactivating the enzyme of the microbe .Beta tumerone cannot deactivate the enzyme. Thus this study could explain that the presence of Curcumin and Cuminic aldehyde provided the medicinal values to *Curcuma aromatica* against Skin disease causing bacteria *Streptococcus equi*.

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Table 1. Results of CDocking of Phytochemicals with Acetoacetyl-CoA reductase(Receptor)

SL NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Curcumin	43.9041	57.42	13.5159
2	Cuminyl alcohol	25.1047	25.8773	0.7726
3	Cuminic aldehyde	24.2864	26.0722	1.7858
4	Camphene	-43.8548	20.9444	64.7992
5	Borneol	-31.1996	24.1321	55.3317
6	Beta-pinene	-1.0983	20.4536	21.5519

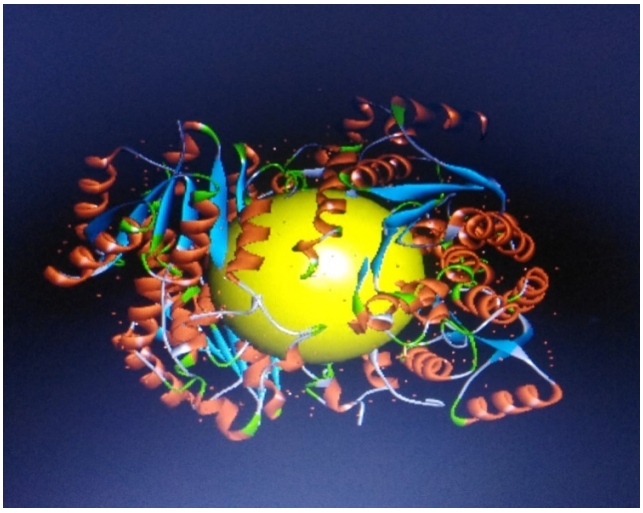

Figure 1: Active Site of Acetoacetyl-CoA reductase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Eclipta alba* against UTI

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Eclipta alba* plant extract is used to cure urinary tract infection (UTI). The plant extract contains different phytochemicals. UTI ismainly caused by *Escherichia coli* (E.coli).One of the key enzymes involved in its biochemical pathway isglycerol-3-phosphate dehydrogenase.The molecular docking of the phytochemicals with the enzyme galactitol-1-phosphate 5-dehydrogenasewas studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.The valuesfor both the parameters indicated that out of different phytochemical of *Eclipta alba* the *pentadecane* can deactivate the glycerol-3-phosphate dehydrogenase enzyme thereby interrupting the life cycle of *E.coli*

Keywords:- Phytochemical, Biovia, Discovery studio, *Eclipta alba, E.coli*

INTRODUCTION

In modern times, life is fast paced, comfortable, readymade, stressful and unhealthy than that of olden days. Less physical activity, sedentary jobs, bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.Modern medicine has become far more advanced in treating illnesses that couldn't be detected in the ancient times.

Nature, the master of craftsman of molecules created almost an inexhaustible array of molecular entities. It stands as an infinite resource for drug development.Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010).Natural products have been the backbone of pharmaceutical throughout the globe.The Medicinal plants have provided mankind a large variety of potent drugs to eradicate infections and suffering from diseases (NatProd Ind J.

2016).Medicinalplants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals ,which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc.(Ullah N., et al.2011). About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants ,(Arulselvan , et al .2013). Many plants also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Plants have been a part of our lives since our existence and have been used for various medicinal purposes . Medicinal plants and herbs like turmeric, ginger, basil leaves, mint and cinnamon are commonly used in Indian dishes and they offer several health benefits. Cold and flu, relieve stress, better digestion, strong immune system and the list is simply endless. Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb coca is the source for cocaine which is act as Local anaesthetic. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010) . Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Eclipta alba, also known as Bhringraj, is a small branched perennial herbaceous plant along with a history of traditional medicinal uses in various countries especially in tropical and subtropical regions of the world.*Eclipta alba* belongs to family *Asteraceae*. *Eclipta alba* extract is used to cure disease like UTI,It is also an effective medicine for skin diseases, cough, asthma, eye disorders and diseases related to any part of the head .The plant to have diverse medicinal values and use it commonly for treatment of gastrointestinal disorders, respiratory tract disorders (including asthma), fever, hair loss and graying of hair, liver disorders (including jaundice), skin disorders, spleen enlargement, and cuts and wounds.*Eclipta alba* is known to contain phytochemicalslike6,10,14-trimethyl-2-pentadecon,7,11-Dimethyl-3-methylene1, Echinocysticacid,heptadecane,octadea-9-enoic acid ,phytol etc. There is high possibility that these phytochemicals play a major role in curing UTI. However, there is no report identifying the specific phytochemical responsible to cure UTI.

Escherichia coli was the most common etiological agent of UTI (Farajnia S, et al. Int J Infect Dis. 2009.)*E.coli* are rod shaped Gram negative bacteria and are about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm^3(wiki).It belongs to family Enterobacteriaceae. In fact, 75% to 95% of urinary tract infections are caused by E. coli.A urinary tract infection (UTI) is an infection in any part of your urinary system — your kidneys, ureters, bladder and urethra.Most infections involve the lower urinary tract — the bladder and the urethra.UTIs are usually caused by *E. coli,* bacteria that are normally found in the digestive tract and on the skin around the rectal and vaginal areas. When the bacteria enter the urethra, they can make their way up into the bladder and cause an infection. The urinary tract is a common source of infection in children and infants and is the most common bacterial infection in children < 2 years of age, both in the community and hospital setting. (Singapore Med J. 2016 Sep; 57(9): 485–490.)This study focuses on the identification of the phytochemical of *Eclipta alba* responsible to cure UTI caused byE.coli.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed thatEclipta alba contains phytochemicalslike6,10,14-trimethyl-2-pentadecon,7,11-Dimethyl-3-methylene1, Echinocystic acid,heptadecane,phytol,octadea-9-enoic acid etc. It has already been established that *Eclipta alba* belonging to Asteraceaefamily has potential to help controlling UTI. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of UTI.

Enzyme found in *E.coli*

It has been reported that UTI can causeas a result of *E.coli* infection.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *E.coli*.bacteria. It has been found that galactitol-1-phosphate 5-dehydrogenaseenzyme have very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Eclipta alba* plant were downloaded from the website (PUBCHEM). The protein database code of thegalactitol-1-phosphate 5-dehydrogenaseenzymewas identified from the website (RCBS PDB). The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu.Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”.The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction waschosen based on a high positive value of -CDOCKER energy and small difference between -CDOCKER energy and -CDOCKER interaction energy(OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala)

Table 1 shows that Pentadecane interaction has the positive value of -CDOCKER energy (27.7246) and minimum value of the difference (2.3684) between - C DOCKER interaction energy and - C DOCKER energy followed Heptadecane. Thus the results indicated that the pentadecane and6,10,14-trimethyl-2-pentadecon can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of*Escherichia coli.* HerePhytol, 7,11-Dimethyl-3-methylene1found to be very less effective withgalactitol-1-phosphate 5-dehydrogenase

and Echinocystic acid cannot interact withgalactitol-1-phosphate 5-dehydrogenase.Thus the key phytochemicals preventing UTI caused by E.coli is Pentadecane.

CONCLUSION

It was previously known that *Eclipta alba* plant has medicinal action against UTI. UTI caused by *E.coli*. Using Discovery studio module of Biovia software, moleculardocking operation was performed to identify the phytochemical (7,11-Dimethyl-3-methylene1,Echinocysticacid,heptadecne,octadea-9-enoic acid , phytol) which can havesignificant interaction with the vital enzymegalactitol-1-phosphate 5-dehydrogenase of microbes. It was found that Pentadecane and Heptadecane can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe while other phytochemicals were found to deactivate the enzyme in a small extent but echinocystic acid cant deactivate the enzyme of *Escherichia coli*.Thus, this study could explain that the presence of pentadecane provided the medicinal values to *Eclipta alba* against UTI caused byE.coli, while other phytochemicals also inhibit metabolism of *Escherichia coli* in a small extent.

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Table 1: Results of Cdocking of Phytochemicals with galactitol-1-Phosphate 5-Dehydrogenase (Receptor).

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Pentadecane	27.7246	30.093	2.3684
2	Heptadecane	31.37	37.5063	6.1363
3	6,10,14-trimethyl-2-pentadecon	20.5632	31.5975	11.0343
4	octadea-9-enoic acid	22.7573	34.7963	12.039
5	Phytol	-6.03838	32.3545	38.39298
6	7,11-Dimethyl-3-methylene-1	-38.5401	19.9615	58.5016
7	Echinocysticacid	Failed	Failed	NA

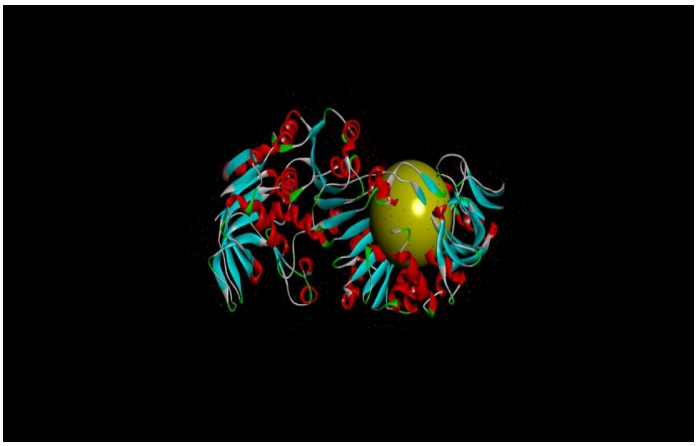

Fig. 1. Show the active site of the galactitol-1-phosphate 5-dehydrogenase.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Lemon grass* against Sinusitis

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ABSTRACT

Phytochemicals are compound that are produced by plant (“ Phyto” means “plant”). Phytochemicals are beginning naturally in many plants and their using up in fruits and vegetables is generally belived to provide beneficial health effect. Phytochemicals are non-nutritive compounds realize from plants [1]. It has been reported that *Lemon grass* plant extract is used to cure sinusitis. The plant extract contains different phytochemicals. Sinusitis is caused by *Streptococcus pneumoniae sp..*One of the key enzymes involved in its biochemical pathway is3-oxoacyl-acyl-carrier- protein reductase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio.The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive valuesfor both the parameters indicated that out of different phytochemical Valeric acid can effectively deactivate the 3-oxoacyl-acyl-carrier – protein enzyme thereby interrupting the life cycle of *Streptococcus pneumoniae.*

Keywords:- Phytochemical, Biovia, Discovery studio, *Lemon grass, Streptococcus pneumoniae*

INTRODUCTION

From Vedic era, it has been proved that plant can cure diseases and the most common source for medicines and even now a day it remains as one. Phytochemical, the non-nutritive part of plant are responsible for the curing of the disease .Every plant possesses a number of phytochemicals, varying in quantity. Some may affect the targeted disease while other simply don’t. This study focuses on determining the specific phytochemical responsible for controlling the targeted disease using molecular docking process of the Bioviasystem .A bygone era , life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad

eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals ,which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc.(Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants . Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Limon grass belongs to family poaceae. Lemon grass leaves extract is used to cure disease like sinusitis. Lemon grass is known to contain phytochemicals likexanthones,citral,quinine,apignin, valeric acid , aspiginin , anthraquinones etc. [2]. There is high possibility that these phytochemicals play a major role in curing sinusitis. However, there is no report identifying the specific phytochemical responsible to cure sinusitis. *Streptococcus pneumoniae* is a commensal organism in the human respiratory tract , meaning that it benefits from the human body , without harming it . However infection by pneumonia, but also bronchitis , otitismedia septicaemia .The contagious period varies and may last for as long as the organism is present in the nose and thoat . A person can no longer spread S. pneumoniae after taking the proper antibiotic for 1-2 days .S. pneumoniae is the most common cause of community acquired pneumonia, bacteremia as well as an important cause of sinusitis. Symptoms of Sinusitis are sinus pressure, headache, inflammation of ear , mouth breathing , phlegm pus , sleeping difficulty , or thoat irritation[3] . A group of bacteria belonging to genus Streptococcuspnemonioe generally cause sinusitis. They are gram positive, lancet shaped cocci bacteria.Streptococcuspnemonioe infection is a common bacterial disease that affects the lungs, ear, bloodstream infection sinus. Humans become infected most frequently through contaminated the coughing from infected person, saliva .

This study focuses on the identification of the phytochemical of *Lemon grass* responsible to cure sinusitis caused by *Streptococcus pneumoniae sp.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Lemon grass* contains xanthones, valeric acid ,citral,quinine,aspiginin , anthraquinones etc.It has already been established that *Lemon grass* plant belonging to Poaceae family has potential to help controlling sinusitis.Lemon grass contains various bioactive compounds that impart medicinal value . It has traditionally used to remediate a medical properties.This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of sinusitis.

Enzyme found in *Streptococcus pnemonioe*

It has been reported that sinusitis can causeas a result of *Streptococcus pneumoniae sp.*infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Streptococcus pneumoniae sp.*bacteria. It has been found that 3-oxoacyl-acyl-carrier-protein(protein database code2Z6J) which is a major enzyme .It has been reported that this specific chemical is involved in lipid metabolism, arachidonate biosynthesis and palmitate biosynthesis (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Lemon grass* plant were downloaded from the website. The protein database code of the 3-oxoacyl-acyl-carrier-proteinenzymewas identified from the website. The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu.Molecular docking was done using the CDockerprotocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the 3-oxoacyl-acyl-carrier-protein enzyme. It appears as light greencolor. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy.The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The

criteria for best interaction was chosen based on a high positive value of -CDOCKER energy small difference between-CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that 3-oxoacyl-acyl-carrier-protein enzyme interaction has the high positive value of -CDOCKER energy (22.1507) and minimum value of the difference (2.7358) between - C DOCKER interaction energy and - C DOCKER energy.

Thus the results indicated that effectively deactivate the 3-oxoacyl-acyl-carrier-proteinenzyme thereby interrupting the biological cycle of *Streptococcus pneumoniae sp.*. Higher positive values for indicated that it was the mostactive ingredient against *Streptococcus pneumoniae sp.*. On the other hand, citral and quinine can deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing sinusitis caused by *Streptococcus pneumoniae sp.*

CONCLUSION

Lemon grass is trendy herb that grow thoughout the year . This plant study like a bush of grass and has a lemony fragrance and so the name . it is usedin various cuisines and also for making medicines .It was previously known that *Lemon grass* plant has medicinal action against sinusitis. Sinusitis is caused by *Streptococcus pneumoniae sp.*.This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (quinine, valeric acid ,xanthones,anthraquinones,citral,aspiginin), which can have a significant interaction with the vital enzyme 3-oxoacyl-acyl-carrier-protein of the microbe.It was found that valeric acid , xanthones , anthraquinones and aspiginincan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbequinine and citralwerefound to be not much effective in deactivating the enzyme of the microbe.Thus, this study could explain that the presence of valeric acid , xanthones , anthraquinones,andaspiginin provided the medicinal values to *Lemon grass* against sinusitis caused by *Streptococcus pneumoniae Sp.*.

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Table1. Results of CDocking of phytochemicals with glycerol-3phosphate oxidase 4x9m (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Valeric acid	22.1507	19.4149	2.7358
2	xanthones	17.858	23.3896	5.5316
3	anthraquinones	17.4634	23.5021	6.0387
4	aspiginin	10.4507	51.1149	40.6642
5	quinine	-24.8971	33.1234	58.0205
6	citral	-14.7	23.1645	37.8645

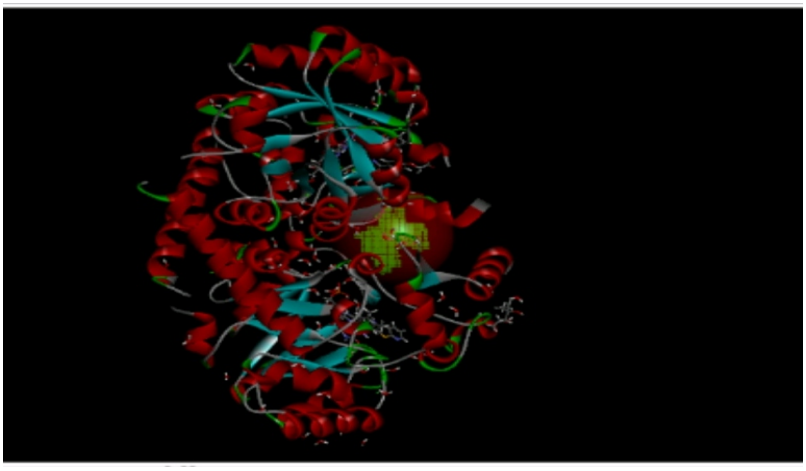

Fig. 1.shows the active site of the 3-oxoacyl-acyl-carrier-protein enzyme.

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Glycyrrhiza glabra* against Shikimate Dehydrogenase of *Haemophilus influenzae* causing Bronchitis

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ABSTRACT

There is an increasing demand for herbal medicines. *Glycyrrhiza glabra* is a plant basically used for preparation of traditional medicine due to presence of some phytochemicals, Phytochemicals are non-nutritive, organic compounds or secondary metabolites produced by plants. Phytochemicals are protective in nature and having various antimicrobial anti oxidant activity. It has been previously established that *Glycyrrhiza glabra* plant extract is used to cure Bronchitis.Bronchitisis caused by *Haemophilus influenzae*. The plant extract contains different phytochemicals.These phytochemicals re play amajor role in curing of bronchitis disease by stop the life cycle of *Haemophilus influenzae*.This bronchitis disease occurs in bronchial tube the air passages that connect the mouth and nose to lungs, in this disease swelling and inflammation of the bronchial tubes occurs [1]. One of the key enzymes involved in its biochemical pathway is shikimate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Catechincan effectively deactivate the enzyme thereby interrupting the life cycle of *Haemophilus influenzae.*

Keywords:- Phytochemical, Biovia, Discovery studio, *Glycyrrhiza glabra*,*Haemophilus influenzae.*

INTRODUCTION

Nature is the major source of medicinal agent; numerous modern drugs are prepared from natural sources. There are several medicinal plants in the nature. Now a day medicinal plants are most require for the preparation of more medicines due to increase of disease level. Plants play an important role in the development of new drugs. The

secondary metabolites or non nutritive componds, bioactive molecules are present in plants are natural compounds and that contain medicinal values of plants and produce definite physiological action on the human body. Thes phytochemicals are used for therapeutic purposes.vegitables and fruits that work as nutrients and in fibres to act against or more specifically contaminated plants. Plants have been used for medicinal purposes before prehistoric period, Ancient unani manuscript Egyptianpapyrus and Chinies writing or describe the use of herb [2]. In ancient days , life was natural, slow, very difficult, peoples are eating traditional food but healthy. Today, in modern days, life is very fast paced, comfortable, readymade, stressfullness, eating many fast food junk food, alcohol etc and unhealthy. Changing work condition, lack of physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has causes some dangerous health hazards like blood pressure, diabetes, obesity etc. If we change our life style and take care our self then we can prevent these lyfestyle related disease. (heinrich Metal., 2010). Plants produce adiverse range of bioactive molecule, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to conform the activity and ti aasertainity the parameteres associated to it. The use of drugs derived from plants areacceleratedby Ehono pharmacologist, botanist, micribologist that is these drugs are are developed for also treatment of infectious diseae. The dis covery of medicinal plantsin different parts of the world is important to medicine and agriculture sector.The plants are the main source of medicines [3]. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011).Medicinal plants play a key role in human health care by curining many disease.(Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items, flavoring. They also played an important role in manufacturing of many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Devi P.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs preparation that prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market.Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants .

Glycyrrhiza glabra belongs to family Fabaceae.It is aherbaceus perennial plant heigh is about 1meter pinnate leaves, the roots are stoloniferous, fruit cotaing many seed. It is native to Mediterian and parts of the South-west Asia. These plants are grown in well drained sandy soil P^H-5.5to 8.2,temp-25^0c . It is also named as licorice.The plant extract is used to cure disease like bronchitis. *Glycyrrhiza glabra* is known to contain phytochemicals like Phloretin, catechin,tannic acid, glycyrrhetinic acid, ,glicyrrhizin,alph-terpin,pectin,rosmarnic acid etc. *Glycyrrhiza glabra* is a herbaceous perennial plant,bearing sweet roots, that are used for therapeutic purposes. The root is known as licorice, licorice used for the tretment of respiratory and digestive disorders and it also help in controlling strss hormones, reducing depression [4].There is high possibility that these phytochemicals play a major role in curing bronchitis. However, there is no report identifying the specific phytochemical responsible to cure bronchitis.

A group of bacteria belonging to family Pasteurellaceae generally cause bronchitis. They are Gram- negative, coccobacillary, faculatively anaerobic pathogenic bacteria. *Haemophilus influenzae* infection is a common bacterial disease that affects the respiratory tractt. These bacteria typically live in animal and human nose, mouth and throat, nosaphyarinx. Humans become infected most frequently through contaminated air wateror food. Bronchotis is aninflamation of the bronchial tubes, the air ways thatcarry air to your lungs. It causes cough that often bring of

mucus and it can also cause shortness of breath,low fever,wheezing, a sre throat,body aches,headache,block nose and sinuses etc. Bronchitis is two types acute or chronic,acute bronchitis usuall clear up but chronic bronchitis is persistent.This study focuses on the identification of the phytochemical of *Glycyrrhiza glabra* responsible to cure bronchitis caused by *Haemophilus influenzae* [5].This study focuses on the identification of the phytochemical of *Glycyrrhiza glabra* responsible to cure bronchitis caused by *Haemophilus influenzae*.

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Glycyrrhiza glabra* contains phloretin ,catechin, tannic acid , glycyrrhetinic acid, glyvrrhizin, alpha-terpin, pectin, rosmarnic acidetc. It has already been established that *Glycyrrhiza glabra* plant belonging to Fabaceae family has potential to help controlling bronchitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of bronchitis.

Enzyme found in *Haemophilus influenzae*

It has been reported that bronchitiscan cause as a result of *Haemophilus influenzae* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Haemophilus influenzae*. It has been found that shikimate dehydrogenase1P74 is involved in phenylalanine, tyrosine, tryptophan biosynthesis and amino acid metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Glycyrrhiza glabra* plant were downloaded from the website. The protein database code ofshikimate dehydrogenase the enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of shikimate dehydrogenaseenzyme.It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019 , 56(2), 111-121)..

Table 1 show that shikimate dehydrogenase and catechin interaction has the highest positive value of -CDOCKER energy (30.328) and minimum value of the difference (9.1909) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that catechin and harmalolcan effectively deactivate the enzyme thereby interrupting the biological cycle of Haemophilus influenza. Higher positive values for indicated that it was the most active ingredient against Haemophylus influenza.On the other hand, alpha terpineol and geraniol can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Cornstarch cannot interact withshikimate dehydrogenase. Thus, the key phytochemicals preventing bronchitis caused by *Haemophilus influenza* are catechin and harmalol.

CONCLUSION

It was previously known that *Glycyrrhiza glabra* plant has medicinal action against bronchitis. Bronchitis is caused by *Haemophilus influenzae*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical phloretin, catechin, tannic acid, glycyrrhetinic acid, glycyrrhizin, alpha-terpin, pectin,rosmarnic acid, which can have a significant interaction with the vital enzyme shikimate dehydrogenase of the microbe. It was found that catechin and harmalolcan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha terpineol and geraniol were found to be not much effective in deactivating the enzyme of the microbe. Cornstarch cannot deactivate the enzyme. Thus, this study could explain that the presence of Catechinand harmalol provided the medicinal values to *Glycyrrhiza glabra* against bronchitiscaused by *Haemophilus influenzae*.

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Table 1. Results of CDocking of phytochemicals with shikimate dehydrogenase(receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Catechin	30.3286	39.5195	9.1909
2	Alpha terpineol	-6.4405	22.6986	29.1391
3	Geraniol	-18.7809	25.6952	44.4761
4	Cornstarch	Failed	Failed	NA
5	Harmalol	11.1498	23.2896	12.1398

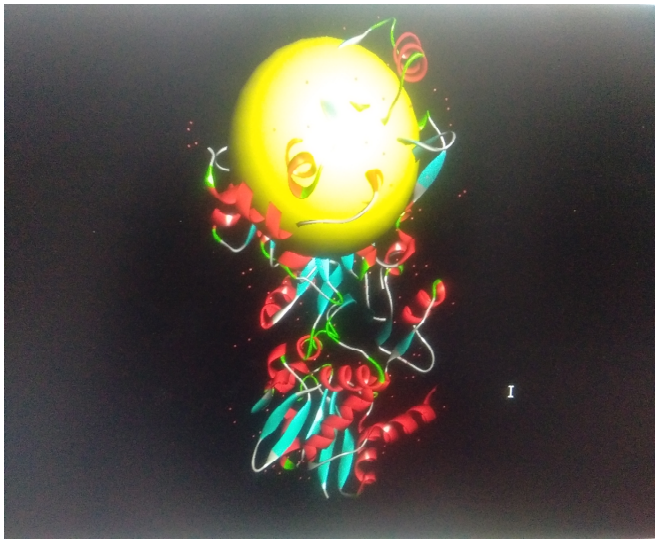

Figure 1. Active site of shikimate dehydrogenase enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemical from *Glycyrrhiza glabra* against Histidine tRNA Ligase of *Haemophilus influenzae* Causing Sore Throat

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Glycyrrhiza glabra* extract is used to cure Sore throat. The plant extract contains different phytochemicals. Sore throat is caused by *Haemophilus influenzae.* One of the key enzymes involved in its biochemical pathway is Histidine Metabolism. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals some can effectively deactivate theHistidine tRNA ligase enzyme thereby interrupting the life cycle of *Haemophilus influenzae*.

Keywords:- Phytochemical, Biovia, Discovery studio, *Glycyrrhiza glabra, Haemophilus sp.*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyles related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010).The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body; these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from

any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants; the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness (Arulselvan, et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R. 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* (Sahoo N.et al, 2010).

Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Glycyrrhiza glabra belongs to family Fabaceae. It is a perennial herb and has been used as a flavouring agent.*Glycyrrhiza glabra* extract is used to cure disease like Eczema, Sore throat, Conjunctivitis, Ulcer, Bronchitis. *Glycyrrhiza glabra* is known to contain phytochemicals like Isocoumarin, Harmalol, Geraniol, Pectin,Liquiritin, Cornstarch etc. There is high possibility that these phytochemicals play a major role in curing Sore throat. However, there is no report identifying the specific phytochemical responsible to cure Sore throat. A group of bacteria belonging to genus Haemophilus generally cause Sore throat. It covers and protects the voice box and wind pipe(trachea) also it is a nonmotile Gram negative bacterium. They are found in the human nose and throats.This study focuses on the identification of the phytochemical of *Glycyrrhiza* responsible to cure Sore throat caused by *Haemophilus influenzae*.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works shows that *Glycyrrhiza glabra* contain Tanic acid, Cortison, Sucrose, Cholesterol, Harmalol, Glycyrrhizin and other phytochemicals. It has already been established that *Glycyrrhiza glabra* belonging to Fabaceae family has potential to help controlling Sore throat. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Sore throat.

Enzyme found in *Haemophilus influenzae*

It has been reported that Sore throat can cause as a result of *Haemophilus influenzae* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Haemophilus*. It has been found that Histidine tRNA ligase (protein database code 3RAC) is present.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Glycyrrhiza glabra* were downloaded from the website. The protein database code of the Histidine tRNA ligase enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “–CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULT AND DISCUSSION

Fig. 1 shows the active site of Histidine tRNA ligase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and ubiquinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019, 56(2), 111-121).

Table 1 show that Histidine tRNA ligase-Isocoumarin interaction has the highest positive value of -CDOCKER energy (15.5847) and minimum value of the difference (6.3128) between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that Isocoumarin can effectively deactivate the enzyme Histidine tRNA ligase thereby interrupting the biological cycle of *Haemophilus influenzae*. Higher positive values forIsocoumarin indicated that it was the most active ingredient against *Haemophilus influenzae*.On the other hand, Harmalol, Geraniol, Pectin can deactivate the enzyme to a small extent. Liquiritin & Corn starch cannot interact with Histidine tRNA ligase. Thus, the key phytochemicals preventing Sore throat caused by *Haemophilus influenzae* are Isocoumarinand Harmalol.

CONCLUSION

It was previously known that *Glycyrrhiza glabra* plant has medicinal action against Sore throat. Sorethroat is caused by *Heamophilus influenza*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Isocoumarin, Harmalol, Geraniol, Pectin, Liquiritin, Cornstarch), which can have a significant interaction with the vital enzyme Histidine tRNA ligase of the microbe. It was found that Isocoumarin and Harmalol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Geraniol, Pectinwere found to be not much effective in deactivating the enzyme of the microbe.Liquiritin& Cornstarch cannot deactivate the enzyme. Thus, this study could explain that the presence of Isocoumarin and Harmalol provided the medicinal values to *Glycyrrhiza glabra* against Sore throat caused by *Haemophilus influenzae*.

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5.DeviP.R, 2014
6.Sahoo N.et al, 2010
7.Iris F. F. et al, 2011
8. OP Brinda1, Deepu Mathew, MR Shylaja , P Sangeetha Davis , K Anita Cherian , PA Valsala, Isovaleric acid and ubiquinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, International Research Journal of Pharmacy-ISSN 2230-8407 2019, 56(2), 111-121.

Table 1. Results of Cdocking of Phytochemicals with Histidine tRNA Ligase (Receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Isocoumarin	15.5847	21.8975	6.3128
2	Harmalol	4.05989	15.4736	11.41371
3	Geraniol	-61.8005	-2.24027	59.56023
4	Pectin	-96.6603	-29.6593	67.001
5	Liquiritin	NA	NA	NA
6	Cornstarch	NA	NA	NA

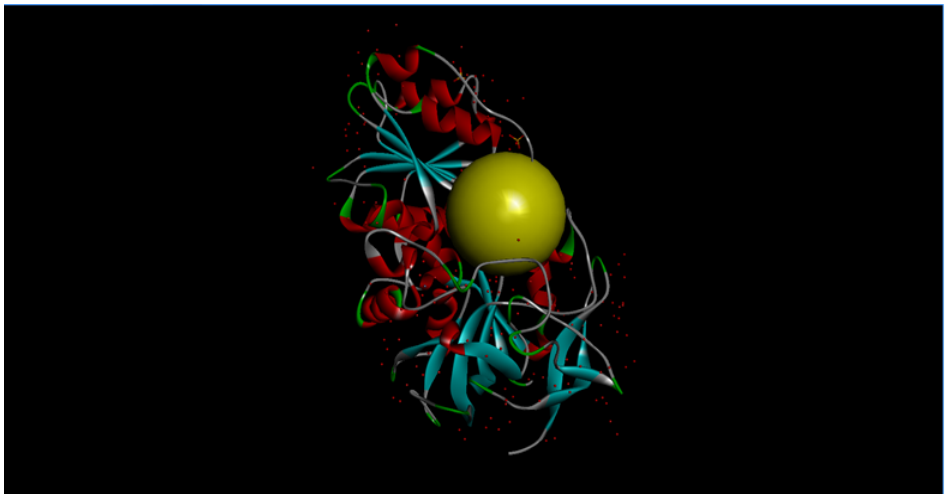

Figure 1. Active Site of Histidine tRNA Ligase Enzyme.

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Murraya koenigii* against Diarrhea

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ABSTRACT

Phytochemicals are the chemical substance produced by the plants and they have biological activities. As we know that *Murraya koenigii* plant extract is used to cure diarrhea. Diarrhea is caused by *Escherichia coli.* Diarrhea is one of the most common health complaints. It can range from mild, temporary conditions, to a potentially life-threating one. (macGill.M,(2017)November, medical news today).one enzyme is taken which is involved in its biochemical pathway is shikimate dehydrogenase which has a protein database code 1NYT. Then molecular docking is done with the phytochemicals of the plants with the enzyme was studied using Biovia Discovery Studio. The stability of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.

Keywords:- Phytochemical, Biovia, Discovery studio, *Murraya koenigii , Escherichia coli.*

INTRODUCTION

Medicinal plants are utilized for primary and traditional healthcare system since very long period of time. Indigenous culture used herbs for healing rituals, while other developed traditional medical system such as Ayurveda which herbal therapies were used systematically. While in this modern time many health hazards faced by peoples due to stress, eating unhealthy food and pollution which cause diseases like diabetes, dysentery, high blood pressure etc. Medicinal plants have always shown their key role in human life for curing diseases. Medicinal plants usage is a result of the many years of struggles illnesses due to which man learned to pursue drugs in barks, seed, fruit bodies and the other parts of the plants. Leaf, roots, flowers, bark and seeds specific extract is used of a plant to cure against the diseases (Jain.V., 2012). Mostly medicinal plant roots are highly anti-inflammation, anti-feedant, anti-microbial properties to fight against the disease (Srivastava et al., 1993). Plants with demonstrated properties of anti-oxidant, anti-inflammation, anti-diabetic and anti-microbial have received research attention.

Curry leaves or *Murraya koenigii* belongs to family Rutaceae. Its leaves extract are used to cure many diseases like dysentery, diarrhoea and eczyma etc. Curry leaves are rich in carbohydrates, fibers, calcium, phosphorous, irons and

vitamin A, vit B, vit E, iron and folic acid. It can improve digestion, lowers cholesterol and prevent greying of hair. Curry leaves contains phytochemicals like Alpha pinene, beta caryophyllene, cinnamic acid, ferulic acid, girinimbine, myrcene, nerolidol, sabinene, terpinen-4-ol. Curry leaves extract are used to cure diarrhea.

Diarrhea is one of the most common health complaints. It happens when the bowl movement becomes watery or loose. It is the passage of 3 or more loose or liquid stools per day or more frequently than normal for the individual. It can range from mild, temporary conditions, to a potentially life-threating one. (macGill.M,(2017)November, medical news today). It is passing loose or watery bowel movements 3 or more times in a day (or more frequently than usual). It can be caused due to many health issues like food poisoning, stomach flu, lactose intolerance, food allergies giardia etc.This study focuses on the identification of the phytochemical of *Murraya koenigii* responsible to cure diarrhea caused by *Escherichia coli.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction between the phytochemical of the plant and the enzyme.

List of Phytochemicals

Phytochemicals are produced by plants these are the non-nutritive components which are involved in the biological activity. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.It has already been established that *Murraya koenigii* plant belonging to Rutaceae family has potential to help controlling diarrhoea. List of phytochemical responsible for inhibiting and controlling of diarrhoea are made like Alpha pinene, beta caryophyllene, cinnamic acid, ferulic acid, girinimbine, myrcene, nerolidol, sabinene, terpinen-4-ol.

Enzyme found in *Escherichia coli*

It has been reported that diarrhoea can cause as a result of *Escherichia coli* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Escherichia coli* bacteria. It has been found that shikimate dehydrogenase enzyme (protein data base code. 1NYT) is involved in Propanol degradation, Ethanol fermentation, phenylalanine metabolism, tryrosine metabolism, methionine metabolism, valine metabolism, Tryptophan metabolism, Leucine metabolism (www.brenda-enzymes.org) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Murraya koenigii* plant were downloaded from the website (pubchem.ncbi.nlm.nih.gov). The protein database code of the shikimate dehydrogenase was identified from the website (www.rcsb.org). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY”

were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the alcohol dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a high positive value of -CDOCKER energy and small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).

CONCLUSION

It was previously known that *Murraya koenigii* plant has medicinal action against diarrhea. Diarrhea is caused by *Escherichia coli*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (alpha pinene,betacaryollene, ferulic acid, cinnamic acid, girinimbine, myrcene, nerolidol, sabinene) which can have a significant interaction with the vital enzyme (shikimate dehydrogenase) of the microbe.It was found that cinnamic acid and ferulic acid confirm strong bond with enyme successfully which inhibit the metabolic pathway (Propanol degradation, ethanol fermentation, phenylalanine metabolism, tyrosine metabolism, methionine metabolism, valine metabolism, tryptophan metabolism, leucine metabolism) of microbe. While alpha pinene, beta caryollene, girinimbine, myrcene, nerolidol, sabinene were not found much effective in the activating the enzyme of microbe. Thus this study explains the presence of cinnamic acid and ferulic acid provides efficient medicinal value to murrayakoenigii against dierrhoea caused by *Escherichia coli*.

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Table 1. Results of CDocking of phytochemicals with alcohol dehydrogenase (receptor)

SL NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Alpha pinene	-13.5732	11.7491	25.3223
2	Beta caryollene	-18.9577	18.1491	37.1068
3	Cinnamic acid	15.7675	18.4501	2.6826
4	Ferulic acid	24.083	30.6724	6.5894
5	Girinimbine	-11.9474	10.8144	22.7618
6	Myrcene	-17.6719	15.4039	33.0758
7	Nerolidol	-42.0385	19.8676	61.9061
8	Sabinene	-14.9807	13.8438	28.8245

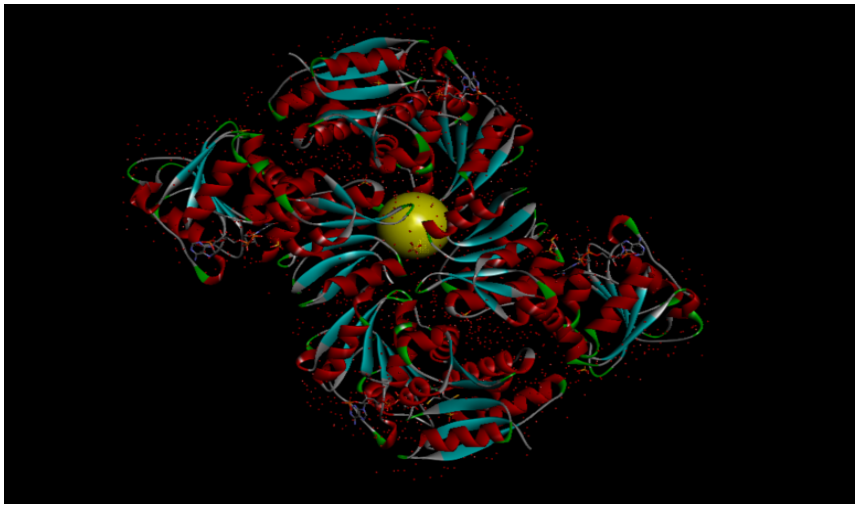

Figure 1. Active site of alcohol dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Curcuma aromatica* against Thymidine Phosphorylase of *Mycoplasma pneumoniae* Causing Bronchitis

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ABSTRACT

Curcuma aromatica is an economically important plant belonging to family Zingiberaceae having both food and medicinal value. Phytochemicals are chemical compounds produced by plants. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been estabilished yet. It has been rumored that *Curcuma aromatica* plant extract is used to cure bronchitis. The plant extract contains different phytochemicals like Alpha tumerone, Beta curcumene, Beta pinine, Beta tumerone, Borneol, Camphene, Cuminic aldehyde, Cuminyl alchohol, Curzerene, D- camphene, D-camphor, Germacrene b. Germacrone, Xanthorrhizo etc.. *Mycoplasma pneumonia* is the bacteria that cause bronchitis. One of the key enzymes involved in its biochemical pathway is pyrimidine metabolism pathway. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals cuminic aldehyde and cuminyl alchohol can effectively deactivate the thymidine phosphorylase enzyme thereby interrupting the life cycle of *Mycoplasma pneumonia*. The plants have been shown to contain bioactive molecules that possess pharmacological properties like anti-inflammatory, antimicrobial, hypocholestraemic, antirheumatic, antiviral, antifibrotic, antivenomous, antihepatotoxic, antidiabetic, antinociceptive, anticancerous, and gastroprotective properties. Agar diffusion method was adopted for determining the antibacterial activity of the extract.

Keywords:- Phytochemical, Biovia, Discovery studio, *Curcuma aromatica, Mycoplasma pneumoniae*.

INTRODUCTION

Life in ancient days was natural, slow, difficult at times but healthy. Today, in modern day to day life, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition due to modern requirements, weak body due to less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce definite physiological actions on the human body; these substances are called phytochemicals, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Curcuma belongs to family Zingiberaceae. Curcuma extract is used to cure disease like Bronchitis [1,2]. *Curcuma aromatica* is known to contain phytochemicals likebeta curcumene, d-camphor, alpha turmerone, beta turmerone, d camphene, P-methoxycinolic acid, Germacrene, curzerene, germacrone, alpha pinene, beta pinene, borneol, alpha terpeniol, myrcene, terpinolene , gama terpinene, alpha copaene, alpha borgamotene,cuminic aldehyde, betabisabolene, Cuminyl alcohol, xanthorrhizol,curcuphenol, beta elemene, zingiberene, linalool , beta farnesene, 8-cinoneole, curzerenone, curcumin, camphene, limonine , beta thujone etc.[1,2,5,3,4]. However, there is no report identifying the specific phytochemical responsible to cure Bronchitis.

A group of bacteria belonging to genus *Mycoplasma* generally cause Brochitis. They are circular Gram negative bacteria. Bronchitis is a common bacterial disease that affects the lungs. Common mild symptoms include sore throat, wheezing and coughing, fever, headache, rhinitis, myalgia and feelings of unease.[6].

This study focuses on the identification of the phytochemical of *Curcuma aromatica* responsible to cure Bronchitis caused by *Mycoplasma pneumoniae*.

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Curcuma aromatica* contains phytochemicals like Alpha tumerone, Beta curcumene, Beta pinine, Beta tumerone, Borneol, Camphene, Cuminic aldehyde, Cuminyl alchohol, Curzerene, D-camphene, D-camphor, Germacrene b. Germacrone, Xanthorrhizoetc. . It has already been established that *Curcuma aromatica* plant belonging to Zingiberzaceae family has potential to help controlling bronchitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Bronchitis.

Enzyme found in *Mycoplasma pnumoniae*

It has been reported that bronchitis can cause as a result of *mycoplasma* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Mycoplasma pneumoniae* bacteria. It has been found that thymidine phosphorylase enzyme protein database code 4LHM, is involved in pyrimidine metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Curcuma aromatica* plant were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/). The protein database code of the thymidine phosphorylase enzyme was identified from the website (https:www.rcsb.org/structure/4LHM). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the thymidine phosphorylase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda1, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).

Table 1 shows that thymidine phosphorylase–cuminyl alchohol interaction has the highest positive value of -CDOCKER energy (13.2302) and minimum value of the difference (1.73) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that cuminyl alchohol can effectively deactivate the thymidine phosphorylase enzyme thereby interrupting the biological cycle of *Mycoplasma pneumoniae*. Higher positive values for indicated that it was the most active ingredient against *Mycoplasma pneumoniae*. On the other hand, alpha tumerone, beta curcumene, curzerene can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Curcumin cannot interact with Thymidine Phosphorylase enzyme. Thus, the key phytochemicals preventing bronchitis caused by *Mycoplasma pneumoniae* are cuminyl alchohol and cuminic aldehyde.

CONCLUSION

C. aromatica is one most useful plant with highly potent pharmacological activities. It was previously known that *Curcuma aromatica* plant has medicinal action against bronchitis. Bronchitis is caused by *Mycoplasma pneumonia*. This study was carried out to provide the theoretical basis of this observation.Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Alpha tumerone, Beta curcumene, Beta pinine, Beta tumerone, Borneol, Camphene, Cuminic aldehyde, Cuminyl alchohol, Curzerene, D-camphene, D-camphor, Germacrene b. Germacrone, Xanthorrhizo) which can have a significant interaction with the vital enzyme (thymidine phosphorylase) of the microbe. It was found that cuminyl alchohol and cuminic aldehyde can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Beta pinine, borneol, camphene, d-camphene, d-camphor were found to be not much effective in deactivating the enzyme of the microbe. Curcumin cannot deactivate the enzyme. Thus, this study could explain that the presence of cuminyl alchohol and cuminic aldehyde provided the medicinal values to *Curcuma aromatica* against Bronchitis caused by *Mycoplasma pneumoniae*.

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Table 1. Results of CDocking of phytochemicals with thimidine phosphorylase (Receptor)

SL NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Alpha tumerone	-33.4195	18.4236	51.83
2	Beta curcumene	-54.4707	13.864	68.33
3	Beta pinine	-59.7991	-19.1105	40.68
4	Beta tumerone	-21.2085	16.3776	37.57
5	Borneol	-123.234	-35.7478	87.49
6	Camphene	-107.079	-14.9089	121.59
7	Cuminic aldehyde	12.606	14.5294	1.92
8	Cuminyl alchohol	13.2302	14.9688	1.73
9	Curzerene	-17.6719	12.1461	29.81
10	D- camphene	-98.6091	-13.3145	85.29
11	D-camphor	-98.532	-25.5309	73
12	Germacrene b	-29.4675	14.0044	43.46
13	Germacrone	-66.3061	15.9475	82.24
14	Xanthorrhizol	-5.88413	17.5022	23.38
15	curcumin	failed	Failed	NA

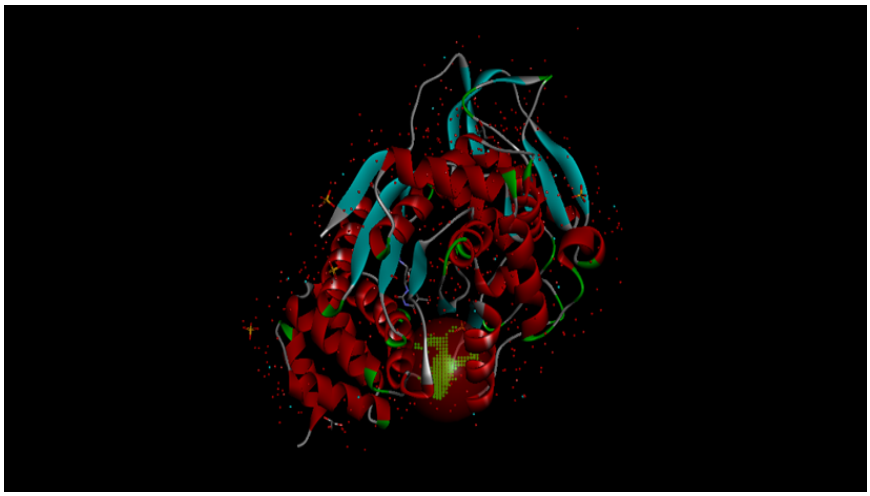

Figure 1. Active site of Thymidine Phosphorylase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Paprika oleoresin (*Capsicum annum* L.) against IMP Dehydrogenase of *Vibrio cholerae* sp. Causing Cholera

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ABSTRACT

Phytochemicals are naturally found in plants that are responsible for treating various diseases by inhibiting the biological cycle of the microbes which are responsible for causing disease. Phytochemicals are biologically active compounds and it is responsible for protecting plants from invasion,disease and infection. It has been reported that paprika oleoresin plant extract is used to cure cholera.Cholera is a bacterial disease which is most frequently transmitted through water sourceswhich is caused by *Vibrio cholerae* sp.The key enzymes involved in it's biochemical pathway of *Vibrio cholerae* sp. is IMP Dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Quercetin can effectively deactivate the enzyme by inhibiting the purine metabolism pathway thereby interrupting the life cycle of *Vibrio cholerae*.

Keywords:- Biovia, Discovery studio,paprika oleoresin,phytochemical,*Vibrio cholera.*

INTRODUCTION

For discovering a drug it is a challenging and long term process. In the year 2014 for developing a new drug the cost was increased by 143% when the study was done by the same organization in the year 2003. This increase was due to failure of the discovered drugs [1]. For a drug discovery project through any of the software the first step is to find

the active photochemical compounds present in the plant. The libraries of millions of compounds are present in pharmaceutical companies but it is quite costly for the large industries for maintenance of the library through high – through put screening. For fulfilling the screening process of millions of compounds with in short period of time or within few days visual screening is a alternative method.When the 3D structure of the target protein is available molecular docking is one of the best known virtual screening method [2] .Molecular docking is a natural process which has the ability to occur within a second when bound to each other in a cell to form a stable complex [3]. The ligandis fit into a binding site in docking method by optimising and combining the variables like steric, electrostatic complementarity and hydrophobic complementarity by estimating free energy binding site.The interaction of two molecules can be made by different ways like Protein proteininteraction or protein and small molecule interaction can be performed[1].

Paprika oleoresin is also refereed as *Capsicum annum* L., Paprika oleoresin is native to Macedonia and grown in different regions.Paprika oleoresin is known to contain phytochemicals likealpha-Terpenol, Apigenin, Beta-carotene, beta-pinene, Camphene, Capsaicin, Cryptocapsin, Cryptoxanthin, Quercetin etc.However, there is no report identifying the specific phytochemical responsible to cure Cholera. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (4). Paprika oleoresin is used as commercial product due to the presence of its colour, and it is used in pharmaceutical as a valuable component. Red pepper due to the presence of its natural colour pigment like capsanthin and capsorubin is a famous variety. The presence of the colour is due to the carotenoidpigment[5,6,7].To improve the product performance such as aroma, taste and colour ground spicy red pepper is used[8,9].Due to the presence of various pharmacological characteristic it has become a part of intrest[10]*Capsicum oleoresin* is used for healing wounds, it helps the body in producing red blood cells,temporary relief in muscle pain or joint pain which is caused by strains,arthritis,bruising sprains,and backaches.

Paprika oleoresin has become a part of interest for its taste improvement and trade qualities of food [11,12,13]. The seed ofPaprika Oleoresinfruit contains valuable fat soluble components such as pigments, flavours and taste agents, vitamins and fattyoil [14,15,16]. Paprika oleoresin is the most popular spices in adding colour and flavor in food.Plants have various types of non –nutritive compounds which are known as phytochemical.There is high possibility that these phytochemicals play a major role in curing cholera. However, there is no report identifying the specific phytochemical responsible to cure cholera.In plants phytochemicals are produced as secondary metabolites. Secondary metabolites are the organic compounds produced in plants they are not directly involved in the growth and development of plant but are essential for the survival of plants. The secondary metabolites are produced by the plants to prevent the plant from infection against other microbes and predators.

The phytochemicals which are produced by plant when taken by humans help to fight against different disease caused by microbes. It helps in curing some disease. The phytochemicals which are present in plants are some times poisonous and cause threat when consumed. And some of the phytochemicals present help in curing disease by traditional methods.This study focuses on the identification of the phytochemical of Paprika oleoresin responsible to cure cholera caused by a bacteria called as *Vibro cholerae*.Cholera is a bacterial disease which is most frequently transmitted through water sources and the causative bacteria for cholera disease is*Vibrio cholerae* sp which results in watery diarrhea which leads to rapid dehydration.The main symptom which can be seen due to this disease is dehydration like vomiting , low blood pressure, wrinkled skin, an rapid heart rate. Cholera can be prevented by taking pure and safe drinking water and non contaminated food; some oral vaccines can also be used for preventing such disease.

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Paprika oleoresin plant belongs to solanaceae family. Published works showed that Paprika oleoresin contains Alpha-terpinol, Apigenin, Beta-carotene, beta-pinene, Camphene, Capsaicin, Cryptocapsin, Cryptoxanthin, Quercetin etc. It has already been established that paprika oleoresin has potential to help controlling cholera. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of *Vibrio cholerae***.**

Enzyme found in Vibrio *cholerae*

It has been reported that cholera can cause as a result of *Vibrio cholerae* infestation. For a microbe to survive various types of metabolic pathways are responsible.Different enzymes performing the metabolic pathways. Brenda enzyme database was used to identify and list different enzymes found in VibrioCholerae. It has been found that IMP Dehydrogenase enzyme protein database code (6MLT) is involved (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

In SBDD one of the most frequent method used is molecular docking .Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the Paprika Oleoresin plant were downloaded from the website (KEGG). The protein database code of the enzyme was identified from the website (BRENDA). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftweres under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the receptor and the ligand. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of IMP Dehydrogenase enzyme.It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy[17].

Table 1 show that Quercetin interaction has the highest positive value of -CDOCKER energy (25.4589) and minimum value of the difference (4.013) between - C DOCKER interaction energy and - C DOCKER energy followed by phytochemical. Thus the results indicated thatphytochemicalQuercetin can effectively deactivate the IMP Dehydrogenaseenzyme thereby interrupting the biological cycle of *Vibrio cholerae* spp. higher positive values forQuercetinindicated that it was the most active ingredient against *Vibrio cholerae*. On the other hand Apigenin , Capsaisin and Beta-pinene can actively participate in deactivating the enzyme which is responsible for causing the disease cholerae having the C-Docker Energy 22.0314 ,4.9364 and 0.06606 respectively. While other phytochemicals present in the plant like Alpha-terpinol, and Camphene can deactive the enzyme which is responsible for causing the disease but in lesser amount having the C-Docker Energy with negative value of -5.25876 and -45.2323. while the

phytocheicals like Beta-carotene and Cryptocapsin can't interact with IMP Dehydrogenaseenzyme. Thus, the best Phytochemicals preventing cholerae caused by *Vibrio cholera* is Quercetin.

CONCLUSION

It was previously known that Paprika oleoresin plant has medicinal action against *Vibrio cholerae*.It is caused by the bacteria *Vibro cholerae*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Alpha-terpinol, Apigenin, Beta-carotene, beta-pinene, Camphene, Capsaicin, Cryptocapsin and quercetin), which can have a significant interaction with the vital enzyme IMP Dehydrogenase of the microbe.It was found that Quercetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe thereby inhibiting the purine metabolic pathway. Thus, this study could explain that the presence of Quercetin provided the medicinal values to Paprika oleoresin against cholera caused by *Vibro cholerae*.

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Table 1. Results of CDocking of Phytochemicals with IMP Dehydrogenase (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKERINTERACTION ENERGY AND - C DOCKER ENERGY
1	Alpha terpinol	-5.25876	22.8101	28.06886
2	Apigenin	22.0314	31.1079	9.0765
3	Beta-carotene	FAILED	FAILED	NA
4	Beta-pinene	0.06606	20.9819	20.91584
5	Camphene	-45.2323	19.6635	64.8958
6	Capsaisin	4.9364	19.9199	14.9835
7	Cryptocapsin	FAILED	FAILED	NA
8	Quercetin	25.4589	29.4719	4.013

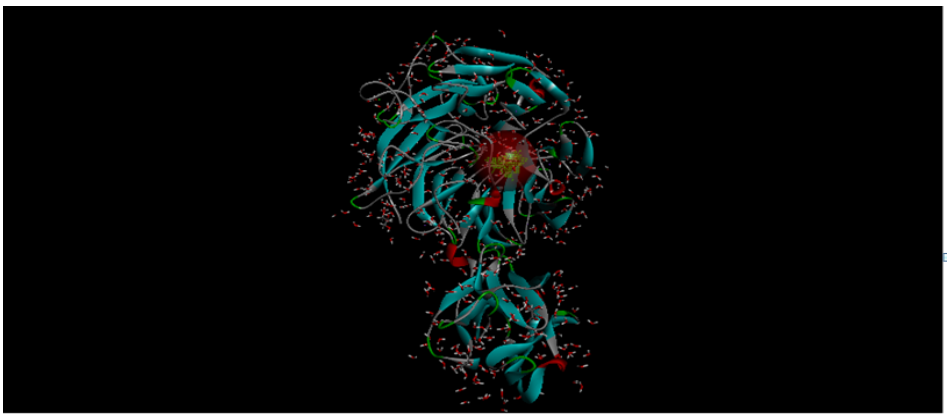

Figure 1: Active site of IMP dehydrogenase Enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Paprika oleoresin* against L-Fucose Isomerase *of Salmonella enterica* causing Typhoid

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ABSTRACT

Phytochemicals (from Greek phyto meaning plant)are chemicals produced by plants through primary or secondary metabolism.These are often called as secondary metabolites and are non nutritive chemical compounds produced by plants via several chemical pathways.It has been reported that *Paprika oleoresin* plant extract is used to cure typhoid. The plant extract contains different phytochemicals. Typhoid is caused by *Salmonella enteric sp..* One of the key enzymes involved in its biochemical pathway is L-Fucose Isomerase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemical Quercetin can effectively deactivate the enzymeL-Fucose Isomerase there by interrupting the life cycle of *Salmonella*.

Keywords:- Phytochemical, Biovia, Discovery studio, Paprica oleoresin,*Salmonella enterica.*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some

chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals ,which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstratedanticancer, antioxidant,anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Paprika extract is used to cure disease like typhoid. Paprika is known to contain phytochemicals like alpha-terpineol, Apigenin, Beta-carotene, beta-pinene, Camphene, Capsaicin, Cryptocapsin, Cryptoxanthinetc, Dihydrocapsaicin acid etc.(NCBI). There is high possibility that these phytochemicals play a major role in curing typhoid. However, there is no report identifying the specific phytochemical responsible to tyohoid.A group of bacteria belonging to genus salmonella enterica generally cause typhoid (NCBI). They are rod shaped, flagellate, facultative aerobic, Gram negative bacterium.salmonella enterica infection (salmonellosis) is a common bacterial disease that affects the intestine. *Salmonella enterica* bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food as well as contact with infective animals, animal feed or humans (NCBI).This study focuses on the identification of the phytochemical of paprika oleoresin responsible to cure typhoid caused by *Salmonella enterica sp..*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that paprik oleoresin contains alpha-Terpineol, Apigenin, Beta-carotene,

betapinene, Camphene, Capsaicin, Cryptocapsin, Cryptoxanthinetc, Dihydrocapsaicin acid acidetc. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of typhoid.

Enzyme Found in *Salmonella enterica*

It has been reported that typhoid can cause as a result of *Salmonella enterica sp.* infestation.Various metabolic cycles have been seen in the bacterial life cychle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in salmonella enterica *sp.* bacteria. It has been found that thiamineL-Fucose Isomerase (protein database code 4C20) is involved in glycerolipid metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the Paprika oleoresin plant were downloaded from the website. The protein database code of the enzymeL-Fucose Isomerase was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the typhoid.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the enzyme. It appL-Fucose Isomerase appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda1, Deepu Mathew2, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121). Table 1 shows that interaction has the highest positive value of -CDOCKER energy (20.081) and minimum value of the difference (6.8533) between -C DOCKER interaction energy and - C DOCKER energy followed by Mycrene..Thus the results indicated that quercetin can effectively deactivate the alcoholL-Fucose Isomerase enzyme thereby interrupting the biological cycle of *Salmonella entericasp.*. Higher positive values for quercetin indicated that it was the most active ingredient against *SalmonellIa entericasp.*. On the other handquercetin,mycrene,alpha-TERPINEOL,sabinene and camphenecan deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Dihydrocapsaicin acid cannot interact with l-fucose isomerase enzyme.Thus, the key phytochemicals preventing typhoid caused by *salmonella enteric sp.*is quercetin.

CONCLUSION

It was previously known that Paprika oleoresin plant has medicinal action against Typhoid.Typhoid is caused by *Salmonella enteric sp.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical), which can have a significant interaction with the vital enzyme (L-fucose isomerase) of the microbe. It was found that quercetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of quercetin provided the medicinal values to Paprika oleoresin against Typhoid caused by *Salmonella enteric Sp.*

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Table 1. Results of Cdocking Of Phytochemicals with L-Fucose Isomerase(Receptor)

Sl No	Ligand	-C Docker Energy	- C Docker Interaction Energy	Difference Between -C Docker Interaction Energy and -C Docker Energy
1	Quercetin	20.081	26.9343	6.8533
2	Mycrene	-12.9537	18.1839	31.1376
3	Alpha – Terpineol	-14.0202	16.2108	30.231
4	Sabinene	-16.2005	16.1721	32.3726
5	Camphene	-58.7799	14.2875	73.0604
6	Dihydrocapsaicin	Failed	Failed	NA

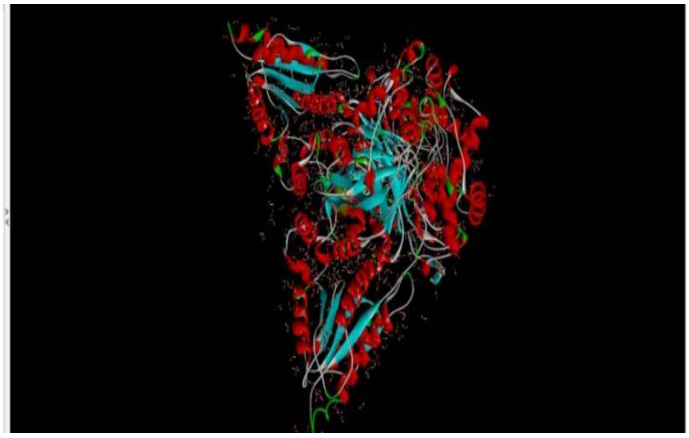

Figure 1. Active Site of L fucose isomerase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Alkanna tinctoria* against Cough

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ABSTRACT

Plants synthesize natural compounds as secondary metabolites or phytochemicals, representing an important source of molecules with a wide range of therapeutic applications. Plant extracts of the *Alkanna tinctoria* plant is believed to cure cough. *Haemophilus influenzae* is reported to cause cough disease. The plant extract of *Alkanna tinctoria* commonly called as ratanjot plant contains different phytochemicals such as 1,8-cineol, 2-hydroxy-3-phenyl-1-4-naphthoquinone, acetylshikonin, alpha terpinyl acetate, anchusin, shikonin . One of the key enzymes involved in its biochemical pathway is shikimate dehydrogenase .The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals, 2-hydroxy-3-phenyl-1-4-naphthoquinone can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Haemophilus influenzae*.

Keywords:- Phytochemical, Biovia, Discovery studio, *Alkanna tinctoria, Haemophilus influenzae*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these

lifestyle related diseases from increasing.The medicinal use of natural products –compounds that are derived from natural sources such as plants , animals or even microorganisms precedes recorded human history probab;y by thousand of years. Throughout our evolution, the importance of natural products for medicine and health has been enormous. Since our earliest ancestors chewed on certainherbs to relieve pain or wrapped leaves around wounds to improve healing, natural products have often been the sole means to treat diseases and injuries. Palaeoanthropological studies at the cave site of Shanidar, located in the Zagros Mountains of Kurdistan in Iraq, have suggested that more than 60,000 years ago, Neanderthals might have been aware of the Medicinal properties of various plants , as evidenced by pollen deposits in one of the graves at the site [1].

Natural products based medicines also flourished in the orient.Charaka Samhita, the first treatise devoted to the concepts and practice of Indian Ayurveda, was written around 900 BC and contains 341 plant derived medicines.The Sushruta Samhita (circa 600 BC) was mainly devoted to surgical practices ,but also described 395 medicinal plants and 57 animal derived products [2].It has only been during the past decades that natural products have taken a secondary role in drug discovery and drug development, after the vast development in molecular biology and biochemistry that made possible to synthesize drugs from natural sources such as plants.

The term phytochemicals refers to the bioactive non nutrient compounds present in the plant-based diet. Numerous lines of evidence indicate that different phytochemicals in the synergy with a range of nutrients, vitamins, minerals, and fiber present in plant-derived foods, possess disease-preventive properties. It has been shown that phytochemicals possess anticarcinogenic and antimutagenic properties, and so, they can play an important role in the lowering of the various types of neoplasia [3,4,5,6,7]. More than 5000 individual phytocemicals have been identified in plant-derived foods, such as fruits, vegetables, and grains and it is estimated that a large percentage of phytochemicals still remain unknown [5,8].Plants are rich source of chemical compounds that are used to accomplish biological activity. Indigenously crude extracts of plants are widely used as herbal medicine for the treatment of infections by people of different ethnic groups .The present investigation was carried out to evaluate the biological potential of *Alkanna tinctoria (*Ratanjot) plant extracts against pathogenic bacteria *Haemophilus influenzae*.

Plant act as reservoirs for wide variety of secondary metabolites including alkaloids, flavonoids,tannins and terpenoids which possess therapeutic properties [9]. The antimicrobial potential of plants have been studied by a large number of researchers across the globe [10,11]. According to World Health Organisation (WHO) in 91 countries there are nearly 2000 medicinal plants [12]. Medicinal plants are still being used by rural communities with increasing popularity for treating or preventing various infections. Among these medicinal plants *Alkanna tinctoria* is under investigation as having several therapeutic uses [13]; however has not been documented for its anti-multi-drug resistant bacterial activity and phytochemicals content.

Ratanjot belongs to family Boraginaceae. Ratanjot plant extract is used to cure disease like cough (NCBI). Ratanjot is known to contain phytocemicals like acetylshikoin, anchusin ,shikonin ,1,8-cineol, 2-hydroxy-3-phenyl-1-4-napthoquinone etc.(NCBI). There is high possibility that these phytochemicals play a major role in curing cough. However, there is no report identifying the specific phytochemical responsible to cure cough.

A group of bacteria belonging to genus Haemophilus is generally found to cause cough. *Haemophilus influenzae* ,a pleomorphic, cocobacillus , gram-negative bacteria is a common commensal of the upper respiratory tract. It is a human-only pathogen that can cause severe disease, including meningitis, pneumonia, and septicemia. *H. influenzae* strains are divided based on the presence or absence of a polysaccharide capsule ; there are 6 encapsulated serotypes (H. influenza serotypes a [Hia], b[Hib], c[Hic], d[Hid], e[Hie], and f [Hif]) and nonencapsulated, nontypeable H. influenza (NTHi) strains. Although Hib strains are considered the most pathogenic, NTHi accounts for a high proportion of all *H. influenza* infections because it causes a notable number of noninvasive infections, such as otitis media and sinusitis , as well as invasive infections [14,15,16,17].This study investigates the identification of the phytochemicals of *Alkanna tinctoria* plant responsible to cure cough caused by the bacteria *Haemophilus influenzae.*

MATERIALS AND METHODS

Software used

For the analysis of medicinal propertries of Ratanjot plant extracts the Discovery studio module of Biovia software (Dassault Systemes of France) was used. Discovery studio is a comprehensive suite of software for simulating small molecule and macromolecule systems and it is developed and distributed by Dassault Systemes Biovia software. Machine learning techniques are utilized by this software to predict the level of molecular interaction between the phytochemicals and the key enzyme present in the metabolic life cycle of the disease causing organism.

List of Phytochemicals

Plants are known to secret various phytochemicals as secondary metabolites to protect them from predators which may include bacteria, viruses, fungi etc., which are known to cause serious threat to the plant. Published works showed that *Alkanna tinctoria* contains phytochemicals like acetylshikoin ,anchusin ,shikonin ,1,8-cineol, 2-hydroxy-3-phenyl-1-4-napthoquinone etc (NCBI) . It has already been established that plant *Alkanna tinctoria* belonging to family Boraginaceae has potential to help controlling cough. This work is focused on identification of the particular phytochemical produced by Ratanjot plant that is responsible for inhibiting and controlling of cough.

Enzyme found in *Haemophilus influenzae*

It has been reported that cough can be caused as a result of *H. influenzae* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival . These metabolic cycles are regulated by different enzymes such as one of the key enzyme is Shikimate dehydrogenase enzyme.Brenda enzyme database was used to identify and list different enzymes found in bacteria *Haemophilus influenzae.* It has been found that Shikimate dehydrogenase enzyme (protein database code-1P74 and E.C. NO-1.1.1.25) is involved in biosynthesis of phenylalanine ,tyrosine and tryptophan, biosynthesis of antibiotics, biosynthesis of amino acids, metabolic pathways and biosynthesis of secondary metabolites (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the plant *Alkanna tinctoria* were downloaded from the website (Pub Chem). The protein database code of the enzyme Shikimate dehydrogenase was identified from the website (www.rcsb.org). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the enzyme Shikimate dehydrogenase. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).Table1 shows that interaction of Shikimate dehydrogenase-2-hydroxy-3-phenyl-1-4 napthoquinone has the highest positive value of -CDOCKER energy (18.3724) and minimum value of the difference (12.81821) between - C DOCKER interaction energy and - C DOCKER energy followed by acetylshikonin, alpha terpinyl acetate , anchusin and shikonin. Thus the results indicated that 2-hydroxy-3-phenyl-1-4-napthoquinone, acetylshikonin, alpha terpinyl acetate, anchusin , shikonin can effectively deactivate the enzyme Shikimate dehydrogenase thereby interrupting the biological cycle of *Haemophilus influenzae*. Higher positive values for 2-hydroxy-3-phenyl-1-4-napthoquinone,acetylshikonin, alpha terpinyl acetate ,anchusin,shikonin indicated that these phytochemicals are the most active ingredient against *Haemophilus influenzae.* On the other hand, 1,8-cineol can deactivate the enzyme to a small extent (negative -CDocker energy but positive difference between –CDocker energy and -CDocker interaction energy). Thus, the key phytochemicals preventing cough caused by *Haemophilus influenzae* are 2-hydroxy-3-phenyl-1-4 napthoquinone, acetylshikonin, alpha terpinyl acetate , anchusin and shikonin.

CONCLUSION

It was previously reported that plant *Alkanna tinctoria* has therapeutical action against cough disease caused by *Haemophilus influenzae*. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (acetylshikoin ,1,8-cineol, 2-hydroxy-3-phenyl-1-4-napthoquinone etc.), which can have a significant interaction with the vital enzyme (Shikimate dehydrogenase) of the microbe. In conclusion ,this study indicates that the phytochemicals (2-hydroxy-3-phenyl-1-4 napthoquinone , acetylshikonin, alpha terpinyl acetate ,anchusin , shikonin) act as potent inhibitors of Shikimate dehydrogenase; being 2-hydroxy-3-phenyl-1-4 napthoquinone as the best inhibitor having highest positive –CDocker energy (18.3724) and minimum value of the difference (12.81821) between - C DOCKER interaction energy and - C DOCKER energy. Other phytochemicals like alpha terpinyl acetate , anchusin ,shikonin , acetylshikonin are also found to be effective in deactivating the enzyme of the microbe since they have positive values of both –CDocker energy and difference between - C DOCKER interaction energy and - C DOCKER energy . 1,8-cineol is found to be not much effective in deactivating the enzyme of the microbe as it is having negative –CDocker energy but positive difference between - C DOCKER interaction energy and - C DOCKER energy. Overall our results encourage use of phytochemicals of the plant *Alkanna tinctoria* in development of therapeutics against cough disease caused by *Haemophilus influenzae.*

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Table 1. Results of CDocking of phytochemicals with Shikimate dehydrogenase (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN –C DOCKER INTERACTION ENERGY AND –C DOCKER ENERGY
1	1,8-cineol	-11.5286	15.6269	27.1555
2	2-hydroxy-3-phenyl-1-4-napthoquinone	18.3724	31.1845	12.81821
3	acetylshikonin.	10.7331	38.1543	27.4212
4	Alpha terpinyl acetate	1.4186	26.5485	25.1299
5	anchusin	2.11042	33.8696	31.75918
6	shikonin	2.11042	33.8696	31.75918

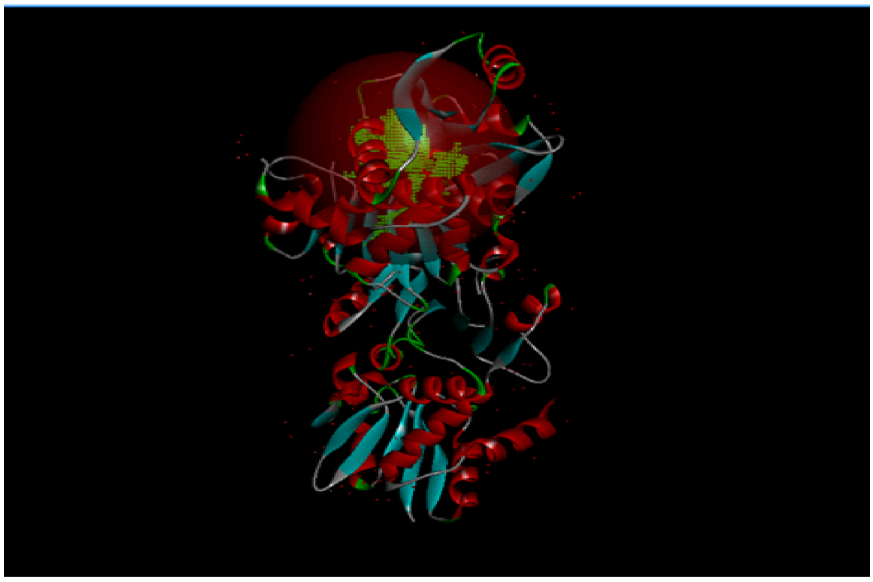

Figure 1: Active site of the Enzyme Shikimate Dehydrogenase

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RESEARCH ARTICLE

In Silico Analysis of Phytochemicals from *Alkanna tinctoria (Ratanjot)* against L-Lactate Dehydrogenase of *Staphylococcus* causing Skin Abscesses

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ABSTRACT

Plant extract are the non-nutritive chemical compounds called as phytochemicals. Generally it considered as the secondary metabolites which is act against competitors, predators or pathogens in plant. It has been reported that *Ratanjot (Alkanna tinctoria)* plant extract is used to cure Skin Abscesses. The plant extract contains different phytochemicals. Skin Abscesses is caused by a bacteria *Staphylococcus aureus.* One of the key enzymes involved in its biochemical pathway is homoserine dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals 1,8-CINEOL, 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin can effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the life cycle of *Staphylococcus aureus*.

Keywords:- Phytochemical, BIOVIA, Discovery studio, *Alkanna tinctoria*, *Staphylococcus aureus*.

INTRODUCTION

The oldest documented medicinal system in the world is siddha medicinal system originating in Tamil Nadu, India. It is the traditional medicine system based on plant[1].The modern society accelerate in a very rapid speed. Due to the population explosion and life is become unhealthy and stressful. Less physical work, over work loading for high demand, less job satisfaction, increase toxic level in food and environment pollution can cause many health hazards

in human. In ancient days, life was slow, totally depends on nature and natural sources. It was so difficult at that time but healthier. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.Plant have provided with all the needs related to Man interm of shelter, clothing, food, flavours and fragrances as not the least, medicines. Plants have formed the basis of sophisticated traditional medicine systems among which are Ayurvedic, unani, Chinese amongst others [2]. The highly diverse plant kingdom, consisting of some 250,000-300,000 species, continues evolution and new adaption to the environment and to protect from pathogens and predators [3]. About 25% of the drugs prescribed worldwide come from plants [4]. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds [5]. Various medicinal plants and their phytoextracts have shown popular household remedies and numerous medicinal properties like traditional healing, antioxidant, antibacterial etc [6]. Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. [7]. Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Ratanjot belongs to family Borages. Ratanjot extract is used to cure disease like Skin Abscesses. Ratanjot is known to contain phytochemicals like 1-8-CINEOL, 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin etc. There is high possibility that these phytochemicals play a major role in curing Skin Abscesses. However, there is no report identifying the specific phytochemical responsible to cure Skin Abscesses.

A group of bacteria belonging to genus *staphylococcus* generally cause Skin Abscesses. They are round shaped Gram positive bacteria. *Staphylococcus aureus* infection is a common bacterial disease that affects the tissue of the body. *Staphylococcus* bacteria typically live in animal and human tissue of the body. Humans become infected most frequently through contaminated bacteria through direct contact. This study focuses on the identification of the phytochemical of *Ratanjot* responsible to cure Skin Abscesses caused by *Staphylococcus aureus.*

MATERIALS AND METHODS

Software Used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these

phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that *Ratanjot* contains 1,8-CINEOL, 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin . It has already been established that *Ratanjot* plant belonging to Borages family has potential to help controlling Skin Abscesses. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Skin Abscesses.

Enzyme found in *Staphylococcus aureus*

It has been reported that Skin Abscesses can cause as a result of *Staphylococcus aureus* infection. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Staphylococcus aureus* bacteria. It has been found that L-lactate dehydrogenase enzyme (protein database code 3D4P) is involved in many metabolism pathway (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Ratanjot* plant were downloaded from the website. The protein database code of the L-lactate dehydrogenase enzyme was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the L-lactate dehydrogenase enzyme. It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmis pentaphylla (Retz.) Correa, 2019 , 56(2), 111-121). Table 1 show that L-lactate dehydrogenase-2-Hydroxy-3-phenyl-1-4-naphtoquinon interaction has the highest positive value of -CDOCKER energy (22.4965) and minimum value of the difference (12.2147) between - C DOCKER interaction energy and - C DOCKER energy followed by these two. Thus the results indicated that 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin can effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Staphylococcus aureus.*Higher positive values for 2-Hydroxy-3-phenyl-1-4-naphtoquinon indicated that it was the most active ingredient against *Staphylococcus aureus.*On the other hand, 1,8-CINEOL can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker

interaction energy). Thus, the key phytochemicals preventing Skin Abscesses caused by *Staphylococcus aureus* are 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin.

CONCLUSION

It was previously known that *Ratanjot* plant has medicinal action against Skin Abscesses. Skin Abscesses is caused by *Staphylococcus aureus*.. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin), which can have a significant interaction with the vital enzyme (L-lactate dehydrogenase) of the microbe. It was found that 2-Hydroxy-3-phenyl-1-4-naphtoquinon can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. 1,8 CINEOL were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of 2-Hydroxy-3-phenyl-1-4-naphtoquinon, acetylshikonin, alpha terpinyl acetate, anchusin and shikonin provided the medicinal values to *Ratanjot* against Skin Abscesses caused by *Staphylococcus aureus.*

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Table 1. Results of CDocking of Phytochemicals with L-lactate Dehydrogenase (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	2-Hydroxy-3-phenyl-1-4 naphthoquinone	22.4965	34.7112	12.2147
2	Acetylshikonin	14.4545	46.8219	32.3674
3	Shikonin	7.59899	39.8859	32.28691
4	Anchusin	7.59899	39.8859	32.28691
5	Alpha-Terpinyl acetate	6.75349	31.9778	25.22431
6	1,8-CINEOL	-1.62812	25.6059	27.23402

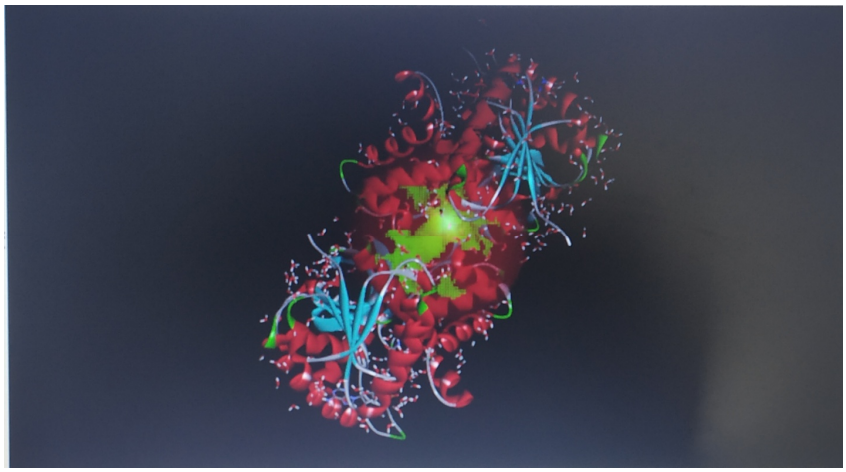

Figure 1. Active site of L-Lactate Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Sandal Wood* against Dysentery

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ABSTRACT

Phytochemical (derive fomgreek word phyto means plant) they are biological active naturally occurring chemical compounds having some health benefits. They are deriving from plants and contain micro nutrient and macro nutrients. Some dietary phytochemicals are present in fruits, vegetables, legumes, wholegrain, nuts, seeds, fungi, herbs and spices. Some common sources of phytochemical are broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries and strawberries etc.[1]. The term phytochemicals refersto plant-derived non-nutritive compounds with therapeutic activities; anticarcinogenic, antimutagenic and antioxidant properties. Based on their chemical structures and charactersticis phytochemicals are classified into six major categories including carbohydrate, lipids, phenolics, terpenoids and alkaloids and other nitrogen containing compounds. It has been reported that *Sandal wood* plant extract is used to cure Dysentry. The plant extract contains different phytochemicals. Dysentry is casued by *Entamoeba histolytica*.One of the key enzymes involved in its biochemical pathway is alcohol dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive valuesfor both the parameters indicated that out of different phytochemicals isoorientin and isorhamnetin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the life cycle of *Entamoeba histolytica*.

Keywords:- Phytochemical, Biovia, Discovery studio, Sandal wood, *Entamoeba histolytica*

INTRODUCTION

Before prehistoric period plants has been used for many medicinal purposes.Medicinal plants also known as medicinal herbs.The use of herbs written inAncient Unanimanuscripts Egyptian papyrus and chiense.Evidence endures that Indian Vaids and European and Mediterranean and Unani Hakims cultures were using herbs for over 4000years as medicine.In dispersion trough ancient civilisation, India has been recognised to be rich respiratory of

medicinal plants[2].From the beginning the human beings have dependent on nature for their common requirement as for the source for medicines ,shelters, food stops ,fragrances ,clothing, flavours and fertilizers. Although the plants produces secondary metabolites, which are responsible for the biological properties of plant species used through out the world [3].The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals ,which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc.(Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy (DeviP.R., 2014).

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the benificial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Sandal wood belongs to family Santalum. It is commonly know as white sandal wood , safedchadan(hindi) and srigandha (sanskrit) ,it is considered to be a admired establishment of plant kingdom woven into culture and heritage of India.Oraised as one of the oldest known perfumery materials having more than 2000 years of ancient history,it has continued its pre-eminence as admired perfumery stuff from antiquity down to modern days[4].It is the second most expensive wood in the world.Both the oil and wood produce a distinctive fragrance that has been used for centuries. Sandalwood.Majority of the people interested in aromatherapy and essential oils are having unique knowledge regarding the relaxing fragrance, native varities of sandalwood in India [3].

Sandalwood like benzene, chlorophyllidea, camphor, chrysin6c, curcumin, ethanol, FADH, phenol, isoorientin, parthenolide,procyanidin,syringic,acid,quercetin7lucoside,tannincacid,isorrhamnetin,vicenin-2,vitexin,FADH,flavon-3-oliso orientin- 7, isovitexin,orientin,orientin-2-o-latoside etc.There is high possibility that these phytochemicals play a major role in curing dysentry. However, there is no report identifying the specific phytochemical responsible to cure dysentry.A group of bacteria belonging to genus generally cause dycentry.It is an infection of the intestinal tract, symptoms include stomach cramps and diarrhea.This study focuses on the identification of the phytochemical of *Sandal wood* responsible to cure Dysentry caused by *Entamoeba histolytica sp.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that *Entamoeba histolytica* contains isoorientin, isorhamnetin, chrysin, benzene, chlorophyllidae,camphor,curcumin,ethanol,FADH,parthenolide,syringicacid,tannic acid,vicenin-2,vitexin, phenol etc .It has already been established that *Sandal wood* plant belonging toSantalaceaefamily has potential to help controlling Dysentry.This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Dysentry.

Enzyme found in *Entamoeba histolytica*

It has been reported that Dysentry can causeas a result of *Entamoeba histolytica sp.*infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found inEntamoebaHistolytica*sp.*bacteria. It has been found that alcohol dehydrogenase enzme (protein data base code 1Y9A) is involved in alcohollipid metabolism (KEGG)and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Sandal wood* plant were downloaded from the website. The protein database code of the alcohol dehydrogenaseenzymewas identified from the website. The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction" menu.Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of the enzyme. It appears as light greencolor. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand.The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in Glycosmispentaphylla (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that alcohol dehydrogenase-isorhamnetin interaction has the highest positive value of -CDOCKER energy (25.4217) and minimum value of the difference (5.816) between - C DOCKER interaction energy and - C DOCKER energy followed

by isoorientin.Thus the results indicated that isoorientin and isorhamnetin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *Entamoeba histolytica.*Higher positive values for isorhamnetin indicated that it was the most active ingredient against *Entamoeba histolytica*.On the other hand, ethanol, benzene, camphor and chrysincan deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy).Digoxin and hexacosanoic acid cannot interact with alcohol dehydrogenase enzyme. Thus, the key phytochemicals preventing Dysentry caused by *isoorientin and isorhamnetin*.

CONCLUSION

It was previously known that *Sandal wood* plant has medicinal action against Dysentry. Dysentry is caused by *Entamoeba histolytica sp.*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemicalchrysin, benzene,chloroohyllidae,camphor,chrysin 6-c,cucrcumin,ethanol,FADH,flavon,isoorientin-7,isorhanetin,isovitexin,orientin,orientin-2-0,phenol,parthenolide,syringic acid,tannic acid,vicenin-2,vitexin), which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe.It was found thatisoorientin and isorhanetincan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Ethanol, benzene, camphor and chrysin werefound to be not much effective in deactivating the enzyme of the microbe. Digoxin and hexacosanoic acid cannot deactivate the enzyme.Thus, this study could explain that the presence of isoorientin and isorhanetin and provided the medicinal values to *Sandal wood* against Dysentry caused by *Entamoeba histolytica Sp.*.

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Table 1. Results of CDocking of phytochemicals with Alcohol Dehydrogenase (receptor)

SL. NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Isorhamnetin	25.4217	31.2377	5.816
2	Isoorientin	14.0652	39.6896	25.6244
3	Chrysin6c	13.3971	36.8657	23.4686
4	Vicenin	-1.98229	44.4213	46.40359
5	Vitexin	8.85689	34.2159	25.35901
6	Flavon	12.369	19.6215	7.2525
7	Syringic acid	Failed	Failed	NA
8	Tanninic acid	Failed	Failed	NA

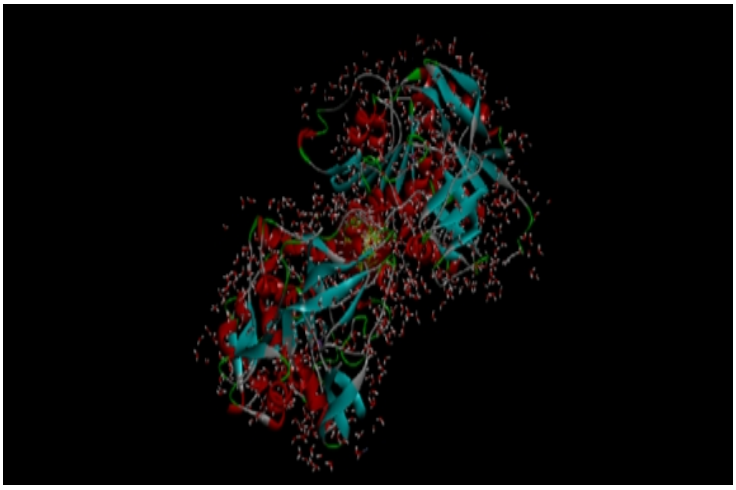

Figure 1. Active site of Alcohol Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Lagerstroemia speciosa* against Dysentery

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Lagerstroemia speciosa* plant extract is used to curedysentery.The plant extract contains different Phytochemicals is dysenterysed by protozoan parasite *Entamoeba histolytica*.One of the key enzymes involved in its biochemical pathway is alcohol dehydroginase.The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy.High positive valuesfor both the parameters indicated that out of different phytochemicalschrysin and hygrine can effectively deactivate the alcohol dehydroginaseenzyme thereby interrupting the life cycle of Protozoan parasite.

Keywords:- Phytochemicals,Discoverystudio,*Lagerstroemia speicosa,Entamoeba histolytica*

INTRODUCTION

In old days life was natural, slow&difficult at time but healthy. Today in modern time life is fast paced, comfortable, readymade, stressful and unhealthy. Changing life conditions, less physical activity, sedentary job Confrtablebutstressful life and bad eating habits expose us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken we can prevent these lifestyle related disease from increasing. Nature has been a source of many medicinal agents for thousands of years and an

impressive number of modern drugs have been derived from natural source. Medicinal plants are the foundation of many drugs. The medicinal value of the plants lies in some chemical substances that produce definite physiological actions on the human body, these substances are called phytochemicals. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti- microbial, anti-diabetes action etc.(Ullah N., et al.2011).Medicinal plants play a key role in human health care (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials fora number of old and new pharmaceutical products.Medicinalplants are the foundation of many drugs prescribed today in modern medicinalsystem. About 25% of modern pharmaceutical drugs have botanical origins, for example the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine& vinblastine from *Catharanthus roseus* & morphine & codeine from *Papaver somniferum* .

Lagerostremia speciosa belongs to family lythraceae. This plant is a tropical flowering tree is one of the most outstanding summer bloomers. In common *Lagerostremia speciosa* is called Queen crape myrtle. This plant is grown in South East Asia, India and the Philippines. It widely cultivated as an ornamental plant in tropical and subtropical areas. Banaba herb is one of the 69 herbal plants promoted by the Philippine department of health (DOH). In Vietnam the plant's young leavs are consumed as vegetables and its old leaves and mature fruit are used in traditional medicine for reducing glucose in blood. The leaves are used in the Philippines as a folk medicine for the treatment of diabetes & kidney diseases. The fruit are used India to cure mout ulcers. The roots are also considered astringent, &seeds have narcotic properties.

Dysentery is an intestinal infection that you can get if you eat food that's been prepared by someone who's get the illness. The causes of dysentery is usually *shigella*, in which case it isknown as *shigellosis* or *Entamoeba histolytica*. Other cases may include certain chemicals, other bacteria, otherprotozoa or parasitic worms. There are 2 types of dysentery.Amoebic dysentery comes from a parasite called *Entamoeba histolytica.*It is more likely to get this kindifyoutravel to a tropical location that has poor sanitation. Second type is Bacillary dysentery comes from bacteria called shigella. Diarrhea from shigella is also called shigellosis.

Dysentery may spread between risk factors include contamination of food and water with feces due to poor sanitation. You can get dysentery if you touch something that has the parasite or bacteria on it, such as toilet handle or sink knob. Swimming in contaminated water such as lacks or pools.The underlying mechanism involves inflammation of the intestine especially of the colon. The symptoms usually show up 1 to 3 dayafter you getinfected. Each type of dysentery has slightly different symptoms. Bacillary dysentery causes symptoms like diarrhea with belly cramps, fever, nausea and vomiting, blood or mucus in the diarrhea. Amoebic dysentery usually doesn't cause symptoms soit leads to more serious problem like liver abscess which is collection of pus in the liver include symptoms such as nausea, diarrhea, belly cramps, weight loss, fever etc.

Efforts to prevent dysentery include hand washing and food safety measures while travelling in area of highrisk, drinking sufficient fluids such as oral rehydration solution is important. Antibiotics such as azithromycin may be used to treat cases associated with travelling in the developing world. Although there is currently no vaccine which protect against shigella infection, several are in development. Vaccination may eventually become a part of strategy to reduce theincidenceand severely of diarrhea, particularly among children in low resource setting.

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.Published works showed that *Lageostremia speciosa* containsAlkaloids, alphaamino acid, gluco corticoids, glycosides saponins, sitosterols and tannins etc.It has already been established that *Lageostremia speciosa* plant belonging to lythraceaefamily has potential to help controlling dysentry. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of dysentry.

Enzyme found in *Entamoeba histolytica*

It has been reported that dysentry can causeas a result of *Entamoeba histolytca* infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found *Entamoebahistolytca*spinbacteria. It has been found that alcohol dehydroginase is involved in glycerolipid metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe.The Discovery studio module of Biovia software was used for identifying molecular interaction and perform molecular docking. In this process first the sdf files for the phytochemicalsfound in the *Lagerstroemia speciosa* plant were downloaded from the website. The protein database code of the glycerol dehydrogenaseenzymewas identified from the website. The active site of the enzyme was identified via “receptor cavity”protocol found under "receptor-ligand interaction"menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig.1 shows the active site of alcohol dehydrogenase it appears as greencolor.CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid- based molecular docking method and optimized for accuracy. The ligand conformations were obtained by molecular Dynamic methods.

-CDOCKERenergywascalculatedbasedontheinternalligandstrainenergyandreceptor-ligand interaction energy.–C DOCKER interaction signifies the energy of the non bonded interaction that exists between the protein and the ligand.The criteria for the best interaction was chosen based on a high positive value of –CDOCKER energyb) small difference between –CDOCKER energy and –CDOCKER interaction energy(OP Brinda, Deepu Mathew, MR Shylaja, P Sabgeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-c are chikungunya virus resistance principles in glycosmispentaphylla (Retz) Correa, 2019, 56(2), 111-121).Table 1 shows that Alphaamino acid has the highest positive value of -CDOCKER energy (20.9346) & minimum value of the difference (1.773) between CDOCKER interaction energy and CDOCKER energy followed by. Thus the results indicated those Alpha amino acids can effectively deactivat the alcohol dehydrogenase enzyme. Higher positive value of Alpha amino acid

indicated that it was the most active ingredient on the other hand saponins, Glycosides1, Sistosterois, Alkaloid 1, and Glucocorticod loosely interact with Alcohol dehydrogenase enzyme.

CONCLUSION

It was previously known that *Lagerstromia speciosa* plant has action against dysentery the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical alkaloid 1, alpha amino acid, glucocorticoid, glycosides 1, saponins, sistosterols which can significant interaction with the vital enzyme (alcohol dehydroginase) of the microbs. It was found that alpha amino acid and saponins can form strong bond with the enzyme successfully.Thus, this study could explain that the presence of alpha amino acid and saponin provided the medicinal value to *Lagerstroemia speciosa* against Dysentery.

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Table 1. Results of CDocking of Phytochemicals with Alcohol Dehydrogenase (receptor)

SL. NO.	LIGAND	-C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	Difference between –C DOCKER interaction energy and –C DOCKER energy
1	Alpha amino acid	20.9346	19.1616	1.773
2	Saponins	4.31847	6.49423	2.17576
3	Glycosides 1	19.8411	25.1827	5.3416
4	Sistosterols	-41.1896	28.3347	12.8549
5	Alkaloid 1	-30.3858	27.5624	57.9482
6	Glucocorticod	-33.5396	32.7977	66.3373

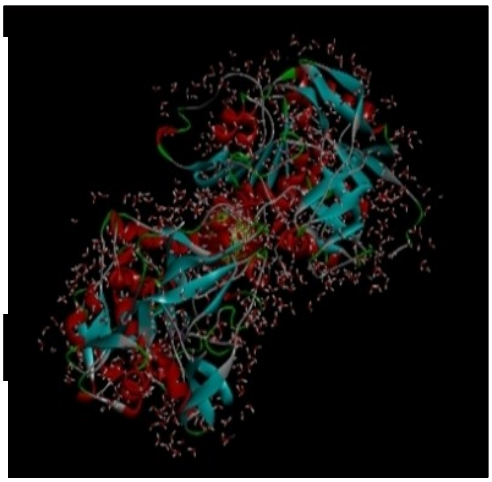

Figure 1: Active Site of Alcohol Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from Tomato Flakes against Alcohol Dehydrogenase of *Entamoeba histolytica* causing Dysentery

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ABSTRACT

Medicinal plants contain many phytochemicals constituents that have protective or disease preventive properties. Phytochemicals are non-nutritive compounds or secondary metabolites obtained from plants. It produces definite physiological action on human body. It has been reported that Tomato flakes extract is used to cure Dysentry because the extract contains different phytochemicals. Dysentry is caused by *Entamoeba histolytica*. Tomato flakes means dried tomato, is a zesty and sweet in taste. Tomato (*Solanum lycopersicum*) is full of vitamin C and lycopene these two things are play a major role it control cacer and heart disease. Tomatoflakes also contain many phytochemicals like kaempfero ,naringenin ,quercetin, etc are play an important role in controlling dysentery.Dysentry is the disease in which infection of the intestine resulting in severe diarrhoea with the presence of blood and mucus in the faeces [1]. One of the key enzymes involved in its biochemical pathway is alcohol dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for the parameters indicated that out of different phytochemicals quercetincan effectively deactivate the enzyme thereby interrupting the life cycle of Entamoeba histolytica.

Keywords:- Phytochemical, Biovia, Discovery studio, Tomato flakes,*Entamoeba histolytica.*

INTRODUCTION

In the last few decades there has been an exponential growth in the field of herbal medicines.It is getting popularized in developing and developed countries owing to its natural originand lesser side effect.Plant derivatives had been employed by population to prevent different kind of diseases.The knowledges of plant properties was acquired by

ancient civilization that passed away fromgeneration to generation untill today. Plant shows wide range of pharmacologicalactivities including anti microbial antiallergesic ,anti oxidants, anticancer, caardiovascular, CNS etc. Traditional systems of medicine continue to be wideused in many sectors. Population rise, inadequate supply of drugs, prohibited cost of treatments, side effect of several synthetic drugs against infetious diseaseshave increased. The use of plant material as asource of medicines for a wide varity of human ailments.In ancient days, peoples are so healthy because life was natural, slow, very difficult and eating traditional food. Today, in modern times, peoples are facing much hazardiou disease due to some reasions like life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.(1) Nature has been a source of medicinal agents for thousands of years (heinrich Metal., 2010). The medicinal plants produced some secondary metabolites, non nutritives substance or organic compound collective known as phytochemical. The phytochemicals not involved in prymary metabolites like growh, develpoment, reproduction etc. these are only involve in protection prevention and compitition. It produces specific physiological actions on the human body, which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011).Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). They also played an important role in manufacturing of many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014).

Medicinal plants are used for manufacturing of many drugs today. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* (Sahoo N.et al, 2010). Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models. (Iris F. F. et al, 2011).

Tomato belongs to familySolanaceae. Tomato flakes extract is used to cure disease like dysentry. Tomato flakesis known to contain phytochemicals likeKaempferol, Naringenin, lycopene, quercetin, phytotene etc.Dehydrated tomato flakes is a sweet and zesty seasoning that absolutely bursts with flavor in their sun dried state. Tomato flakes maintains healthy blood pressure, and reduce blood glucose level.There is high possibility that these phytochemicals play a major role in curing dysentry. However, there is no report identifying the specific phytochemical responsible to cure dysentery [2].

A group of amoebazoanbelonging to family Entamoebidaegenerally cause dysentry. They are spherical or oval shaped, anaerobic, parasitic amoebazoan and Gram negative stain. *Entamoeba histolytica* infection (amebiasis) is a common disease that affects the intestinal tract. Entamoeba histolytica feedson cells in the human colon; it is the cause of amoebic dysentry (bloody diarrhea) as well as colonic ulceration. Dysentry is the disease by which infection of the intestine result in diarrhea with blood, fever,abdominal pain, it is also known as shigellosis. Entamoeba histolytica typically live in animaland human large intestine and are shed through feces. Humans become infected most frequently through contaminated water or food, poor hand washing,swimmimg in contaminated water. The

people who don't have dysentry come in cotact with faecial matter from people who do have dysentry. This study focuses on the identification of the phytochemical of Tomato flakes responsible to cure dysentery caused byEntamoeba histolytica [3].This study focuses on the identification of the phytochemical of Tomato flakes responsible to cure dysentrycaused by *Entamoeba histolytica.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works showed that Tomato flakes contain kaempferol, naringenin, lycopene, quercetn, phytotene etc. It has already been established thatTomatoplant belonging to Solanaceaefamily has potential to help controlling dysentry. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of dysentry.

Enzyme found in *Entamoeba histolytica*

It has been reported that dysentry can cause as a result of *Entamoeba histolytica* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in Entamoeba histolytica. It has been found that alcohol dehydrogenaseprotein database code 1Y9A is involved in (pentose and glucuronate interconversion and glycolipid metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the Tomatoplant were downloaded from the website. The protein database code ofalcohol dehydrogenase the enzyme was identified from the website. The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of alcohol dehydrogenase enzyme.It appears as light green color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the non-bonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda, Deepu Mathew, MR Shylaja, P Sangeetha Davis, K Anita Cherian, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmis pentaphylla* (Retz.) Correa, 2019 , 56(2), 111-121).Table 1 shows that alcohol Dehydrogenase quercetin interaction has the highest positive value of -CDOCKER energy (23.3987) and minimum value of the difference (2.4745) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that quercetin, naringenin and kaempferolcan effectively deactivate the enzyme thereby interrupting the biological cycle of *Entamoeba histolytica*. Higher positive values for indicated that it was the most active ingredient against *Entamoeba histolytica*.On the other hand, lycopene, phytotene can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing dysentrycaused by *Entamoeba histolytica* are quercetin, naringenin and kaempferol.

CONCLUSION

It was previously known that Tomato flakes have medicinal action against dysentry. Dysentry is caused by *Entamoeba histolytica*.This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (kaempferol, naringenin, lycopene, quercetin, phytoten), which can have a significant interaction with the vital enzyme (alcohol dehydrogenase) of the microbe. It was found that quercetin, naringenin and kaempferol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.Lycopene, phytotene were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of quercetin, naringenin and kaempferol provided the medicinal values to Tomato flakes against dysentry caused by *Entamoeba histolytica.*

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Table 1. Results of CDocking of Phytochemicals with Alcohol Dehydrogenase (receptor)

SL. NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Kaempferol	19.8843	24.6955	4.8112
2	Naringenin	20.2663	25.6371	5.3708
3	Lycopene	-79.1059	40.3498	119.4557
4	Quercetin	23.3987	25.8732	2.4745
5	Phytotene	-121.977	38.5355	160.5125

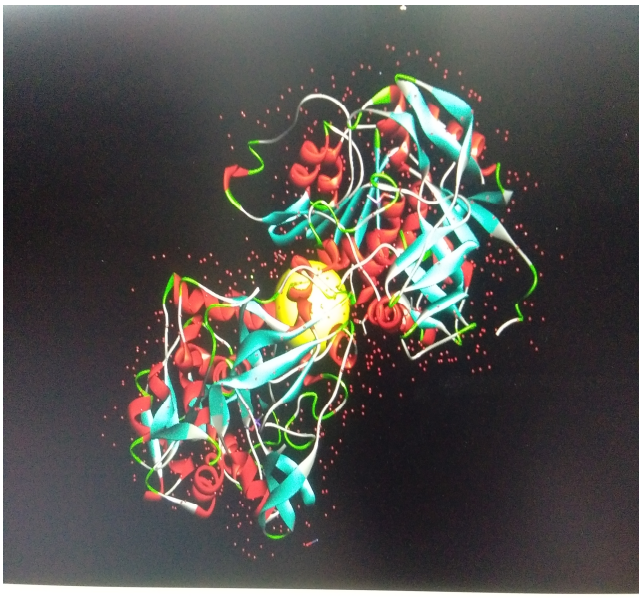

Figure 1. Active site of Alcohol Dehydrogenase Enzyme.

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Trigonella foenum-graecum* against Peptic Ulcer

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that*Trigonella foenum-graecum* plant extract is used to cure ulcer. The plant extract contains different phytochemicals. Ulcer is caused by *Helicobacter pylori.* One of the key enzymes involved in its biochemical pathway is alcohol dehydrogenase.The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals chrysin and hygrine can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the life cycle of *Helicobacter pylori*.

Keywords:- Phytochemicals,Discovery studio,*Trigonellafoenum-graecum*,*Helicobacter pylori*

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called

phytochemicals ,which can be used for therapeutic purpose. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014). Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. (Ullah N., et al.2011). Medicinal plants play a key role in human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants , the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. (Arulselvan , et al .2013). Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, For example, the herb foxglove is the source for digitals and the herb salicin is the source aspirin. Fenugreek (*Trigonella foenum graecum)* is an annual plant in the family fabaceae with leaves consisting of three small abovate to oblong leaflets. It is cultivated worldwide as a semiarid crop. It's seeds and leaves are common ingredients in dishes from the Indian subcontinent.

Fenugreek bark, leaves, flowers, roots, fruits, and seeds (Srivastava P.K., 2014) .FeFenugreek is used as a herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for the distinctive maple syrup smell of fenugreek .Cuboid-shaped, yellow- to amber-coloured fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, dal, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavour.Phytochemicals are compounds that are produced by plants. They are found in fruits, vegetables, grains. They generally have biological activity in the plant host and play a role in plant growth. *Helicobacter* is a bacteria .These germs can enter your body and live in your digestive tract. In some people an infection can lead to stomach cancer. This study focuses on the identification of the phytochemical *of Trigonellafoenum-graecum* responsible to cure ulcer caused by *Helicobacter sp..*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction.

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional medicine.It has already been established that *Trigonella* plant belonging to *fabaceae* family has potential to help controlling ulcer.This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of ulcer.

Enzyme found in *Helicobacter pylori*

It has been reported that ulcer can cause as a result of *Helicobacter sp.*infestation.Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found helicobacter*sp.*bacteria. It has been found that

alcohol dehydrogenase enzyme (protein data base code 3TWO) is involved in chorismate biosynthesis (Brenda) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Trigonellafoenum-graecum* plant were downloaded from the websit. The protein database code of the alcohol dehydrogenase enzyme was identified from the website. The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia softwere under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods conformations were obtained by Molecular Dynamic methods.

CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction energy

CONCLUSION

It was previously known that *Trigonellafoenum-graecum* plant has medicinal action against ulcer. Ulcer is caused by *Helicobacter sp.*. This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical argnine, carpaine, choline, diosgenin, gentianine, gitogenin, histidine , Sarsapogenin, Trigonelline, vitamin E-acetate which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe.

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Table 1. Results of CDocking of phytochemicals with alcohol dehydrogenase (receptor)

SL. NO.	LIGAND	**- C DOCKER ENERGY**	**- C DOCKER INTERACTION ENERGY**	**Difference between - C DOCKER interaction energy and - C DOCKER energy**
1	Arginine	36.2936	38.0427	1.7491
2	L-tryptophan	30.2323	35.1326	4.9003
3	Trigonelline	29.6683	29.3233	0.3451
4	Histidine	27.9857	26.6745	1.3112
5	Gentianine	13.9312	25.7359	11.8047
6	Choline	5.98461	16.9647	10.98809
7	Carpaine	Failed	Failed	NA
8	Diosgenin	Failed	Failed	NA
9	Vitamin -E-acetate	Failed	Failed	NA
10	Sarsapogenin	Failed	Failed	NA
11	Gitogenin	Failed	Failed	NA

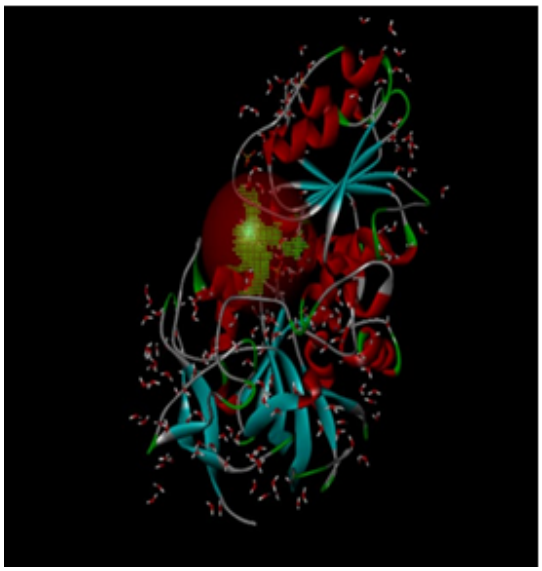

Figure 1: Active site of alcohol dehydrogenase

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RESEARCH ARTICLE

In silico analysis of Phytochemicals from *Lagerstromia speciosa* against Typhoid

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ABSTRACT

The synthesis natural compounds as secondary metabolic or photochemical in plants, representing as important source of molecules with a wide range of therapeutic application. It has been reported that *Lagerstroemia speciosa* plant extract is used to cure Typhoid.The plant extract contains different photochemical such as Alpha amino acid, alkaloids, Gluco-corticoids, Glycosides, Saponins, Tannins and Sitosterols. Typhoid is caused by *Salmonella enteric.* One of the key enzymes involved in its biochemical pathway Phosphogluconate Dehydrogenase (NADP+-dependent, decarboxylating). The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of phytochemicals Alfa amino acids can effectively deactivate the enzyme there by interrupting the life cycle of typhoid.

Keywords:- Phytochemical, Biovia, Discovery studio, *Lagerstroemia speciosa,* Typhoid.

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, less physical activity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us to some dangerous health hazards like blood pressure, diabetes, obesity etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.Nature has been a source of medicinal products for millennia with many

useful drugs developed from plant sources. The thousands of years and an impressive number of modern drugs have been derived from natural source [1]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the human body , these substances are called phytochemicals , The "phyto-" of the word phytochemicals is derived from the greek word phyto,which means plant.Therefore, phytochemcicals are defined as bioactive non-nutrient plant compounds in fruits, vegitables , grains, and other plant foods that have been linked to reducing the risk of major chronic diseases [2].Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant , anti-inflammatory , anti-cancer , anti-microbial, anti-diabetes action etc. [3]. Various models like in-vivo and in-vitro are used for the development of anti-inflammatory drugs [4]. Medicinal plants play a key role in human health care. In world 80% of the population depends up on traditional medicine [5]. Many of the medicinal plants are used as spices and food items. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants [6]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Lagerstromia commoly known as queen crape Myrtle, it is widly distributed in south East Asian country Philippine and India. It's a member of the family Lythraceae. The people of south East Asia used the leaves of *L.speciosa* for the treatment of diabetes mellitus and obesity [7]. *Lagerstromia* is known to contain phytochemicals like Alkaloids, Alfa amino acids, Glucocorticoids, Glycosides, Saponins, Tannins etc. There is high possibility that these phytochemicals play a major role in curing typhoid. However, there is no report identifying the specific phytochemical responsible to cure typhoid.

A group of bacteria belonging to genus *Salmonella* generally cause typhoid. Typhoid infection (*Salmonella enterica*) is faculatative anerobic Gram-negative rod-shaped bacteria generally 2-5 microns long by 0.5-1.5 microns wide motile by peritrichous flagella. *Salmonellae* belong to the family Enterobacteriaceae [8], *S. entrica* are a medically important pathogen for both animals and human. Infection begins with the ingestion of contaminated food or water so that salmonellae reach the intestinal epithelium and trigger gastrointestinal disease. In some patients the infection spreads upon invasion of the intestinal epithelium, internalization within phagocytes, and subsequent dissemination. Humans become infected most frequently through contaminated water or food [9].This study focuses on the identification of the phytochemical of *Alpha amino acid* responsible to cure typhoid caused by *Salmonella Enterica.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemes of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include yeast, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and others as traditional

medicine. Published works showed that *Lagerstroemia speciosa* contains Alkaloids, Alpha amino acids, Glucocorticoids, Glycosides, Saponins, Sitosterols; Tannins etc.It has already been established that lagerstroemia speciosa belonging to Laurelsfamily has potential to help controlling typhoid. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of typhoid.

Enzyme found in *Salmonella enteric*

It has been reported that typhoid can causes a result of *Salmonella enteric* Infestation. Various metabolic cycles have been seen in the yeast life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Salmonella entrica* yeast. It has been found that Phosphogluconate Dehydrogenase enzyme (protein database code 3FWN) is involved in Pentose Phosphate Pathway (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the yeastprotein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Lagerstroemia speciosa* plant were downloaded from the website (www.florajournal.com). The protein database code of the phosphogluconate dehydrogenase *Salmonella enterica* enzymewas identified from the website (https://www.rcsb.org). The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Bioviasoftwere under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The “CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Fig. 1&2 shows the active site of the phosphogluconate dehydrogenase enzyme. It appears as light green colour. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy [9].Table 1 shows that phosphogluconate dehydrogenase-*alpha amino acid* interaction has the highest positive value of -CDOCKER energy (18.9895) and minimum value of the difference (0.819) between - C DOCKER interaction energy and - C DOCKER energy followed by *Alkaloids*. Thus the results indicated that Alpha amino acid can effectively deactivate the Phosphogluconate dehydrogenase enzyme thereby interrupting the biological cycle of *Salmonella enterica*.

CONCLUSION

It was previously known that *lagerstroemia speciosa* plant has medicinal action against Typhoid. Typhoid is caused by *Salmonella enterica.* This study was carried out to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical

(Alkaloids, Alpha amino acids, Gluco-corticoids, Glycosides, Saponins, Sitosterols,Tannins) which can have a significant interaction with the vital enzyme (Phosphogluconate dehydrogenase NADP+-dependent, decarboxylating *Salmonella enterica*) of the microbe. It was found that Alpha amino acid form strong bond with the enzyme successfully. Thus, this study could explain that the presence of Alpha amino acid provided the medicinal values to *Lagerstomia speciosa* against Typhoid caused by *Salmonell enterica.*

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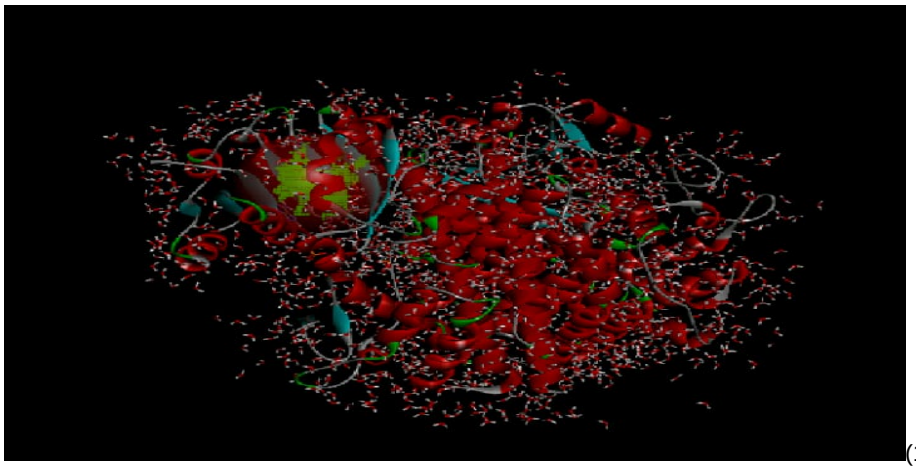
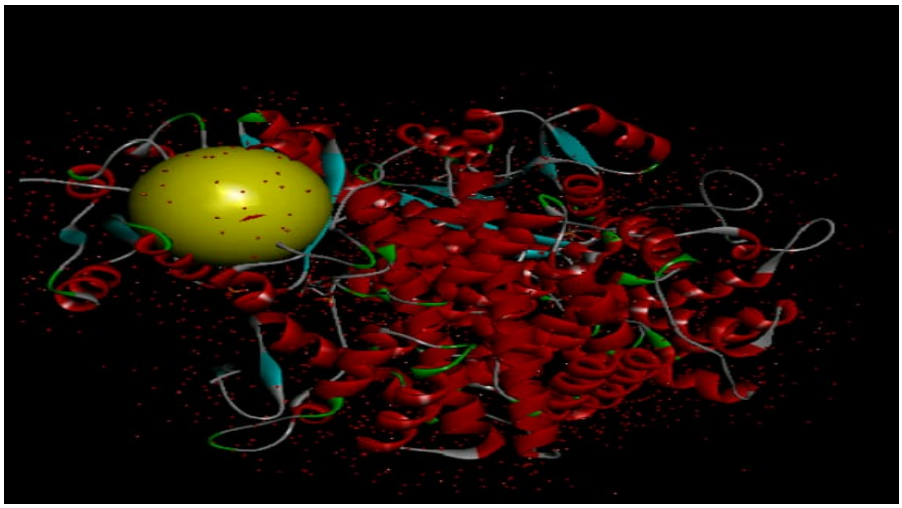
Table 1. Results of CDocking of phytochemicals with Phosphogluconate dehydrogenase (receptor)

SL.NO.	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKERinteraction energy and - C DOCKER energy
1	Alpha amino acid	18.9895	19.8085	0.819
2	Alkaloids	-930.155	-232.254	697.901
3	Saponins	-4.86439	-0.392836	4.471554
4	Glycosides	-170.015	-60.706	109.309
5	Glucocorticoids	FAILED	FAILED	NA
6	Sitosterols	FAILED	FAILED	NA
7	Tannins	FAILED	FAILED	NA



(1)



(2)

Figure 1&2. Active site of Phosphogluconite Dehydrogenase Enzyme.

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REVIEW ARTICLE

A Review on State of Art Cryptographic Algorithms

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ABSTRACT

In today's information technology world, Cryptography plays a vital role in the field of data security. There are a lot of cryptographic algorithms for enciphering and deciphering data but unfortunately none of these is a one stop solution for various issues. For commercialization, properties of such algorithms must be studied so that resource utilization should be optimized. In this paper a comparative analysis is presented among various cryptographic algorithms like AES, DES, RSA, Blowfish, SEA etc. considering cost and performance.

Keywords: Cryptography, Enciphering, Deciphering, AES, DES, RSA, Blowfish, SEA.

INTRODUCTION

This is the era of information and technology where there is a strong competition between storing and exchanging information. As much as it is good; it is that much vulnerable as well. This means that the real time scenario is that there is an intense risk in keeping or communicating with these data or sharing information securely. In order to make data or information secure (i.e. in its original form) we have to do some processing on it to maintain the confidentiality. The afore mentioned processing is of two types i.e. Encryption and Decryption. Encryption is the process of converting the data or information in to such an unreadable form that will not be understood to anyone else even if it may be disclosed, hence during Encryption the Plain text is converted into Cipher text (See Fig. 1). Decryption is the reverse process of encryption. In this process the cipher text is again converted to its original form i.e. the plain text (See Fig. 2). The encryption and Decryption processes are supported by algorithms called Encryption algorithm and Decryption algorithm respectively.

Ciphering & Key

All the above encryption and decryption are known as ciphering. In every ciphering a key is used just like locking and unlocking a lock with a key. According to the key and its use ciphers are categorized in to two major types:

1. Asymmetric key cryptography & Symmetric key cryptography
2. Block cipher & Stream cipher

Asymmetric key cryptography: In this cryptography different keys are used for encryption and decryption (See Fig. 3). Symmetric key cryptography: In this cryptography same key is used for encryption and decryption as well. Hence, it is required to send the key to the receiver for decryption purpose. Block cipher: Here the key act on group of bits of same size so it is named as block cipher (See Fig.5). Stream cipher: Here the key act on continuous bits so it is named as stream cipher (See Fig.6).

Cryptographic Algorithms

To encrypt or decrypt a data or information certain rules are followed commonly called cryptographic algorithm. In this section cryptographic algorithms such as Advanced Encryption Standard (AES), Data Encryption Standard (DES), Blowfish, Rivest-Shamir-Aldeman (RSA) etc are critically reviewed.

Advanced Encryption Standard (AES) Algorithm

It is a symmetric block cipher consists of 128 bit blocks of data and 128 bit key (for 10 round encryption), 192 bit key (for 12 round encryption), 256 bit key (for 14 round encryption). AES has four functions: SubBytes, ShiftRows, MixColumns, and AddRoundKey. These functions provide confusion, diffusion, and XOR encryption to the State. ShiftRows gives diffusion by shifting rows of the State. It treats each row like a row of blocks, shifting each a different amount(i.e. row 0 is unchanged, row 1 is shifted 1 to the left, row 2 is shifted 2 to the left, row 3 is shifted 3 to the left). MixColumns also gives diffusion by “mixing” the columns of the State via finite field mathematics. The SubBytes function gives confusion by substituting the bytes of the State. AddRoundKey is the final function applied in each round. It performs XOR operation on the State with the subkey. The subkey is derived from the key, and is different for each round of AES [5].

Data Encryption Standard (DES) Algorithm

Data Encryption Standard is a block cipher. It processes 64 bit plain text to 64 bit cipher text by using 48 bit key. As it is a symmetric key algorithm so same key is used for encryption and decryption. There are 16 rounds of iteration for encryption and in each round different keys are used. For key generation, circular left shift operation is performed. For encryption mainly xor and swapping operations are carried out. The reverse process of encryption is done for decryption means the order of 16 number of 48 bit key is reversed such that key 16 becomes key 1 and so on [6].

Blowfish Algorithm

In this algorithm the block size is 64 bit; key is of variable length i.e. from 32 bit to 448 bit. It has a feistel structure of 16 rounds. There are five sub keys: one 18 entry p array and four 256 entry s boxes. Generally, XOR and swapping mathematical functions are used here. Decryption is completely same as encryption but the p array is taken in reverse order [7].

Rivest-Shamir-Aldeman (RSA) Algorithm

It is a asymmetric key algorithm. The key size is from 1024 to 4096 bits. Here the major difficulty is factoring two large prime numbers. The prime numbers must be kept secret. It is a very slow algorithm. Here four steps are followed: Key generation, Key distribution, Encryption, Decryption [8].

Scalable Encryption Algorithm (SEA) Algorithm

It is a symmetric low resource high throughput algorithm. It has a feistel structure with variable number of rounds. Here some elementary operations are performed. Those are: Bitwise XOR, Substitution box, word rotation (left), Inverse word rotation, Bit rotation, 2^b modular addition etc [9] [11] [12].

HIGHT Algorithm

It is a block cipher which takes 64 bit plain text as input and gives 64 bit cipher text by using 128 bit key. It has a feistel structure. Simple mathematical functions like xor, 2^8 modular addition, bitwise left shift are used for encryption. Decryption is just reverse process of encryption [3]. A Summary of performance of various cryptographic algorithms is summarized in TABLE 1.

CONCLUSION

In this paper a comparative analysis is done among various cryptographic algorithms and their performances are compared on different environments. For a small memory device Blowfish is to be used due to its low memory consumption. As time consumption is less in case of Blowfish so it can be referred for fastest devices. For highest Avalanche effect AES can be recommended for those systems where confidentiality and integrity is of highest priority. If bandwidth requirement is considered then DES algorithm can be suggested as lowest number of bits used for encryption. AES can be recommended taking a view on cryptographic strength. So there is no such algorithm which is a best choice for a generalized field rather as per requirement the best suited cryptographic algorithm can be choosen to secure the data or information from attacks and unauthorised access.

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Table 1. Comparison of Cryptographic algorithms

Sl. no.	Algorithm	Encryption time	Decryption time	Memory usage	Avalanche effect	Average entropy per byte	No. of bits	CPU usage
1	AES	Moderate	Moderate	Moderate	Highest	Moderate	Highest	More
2	DES	Moderate	Moderate	Moderate	Moderate	Lowest	Lowest	Less
3	Blowfish	Least	Least	Least	Moderate	Highest	Moderate	Moderate
4	RSA	More	More	More	Lowest	Moderate	Moderate	Moderate
5	SEA	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate

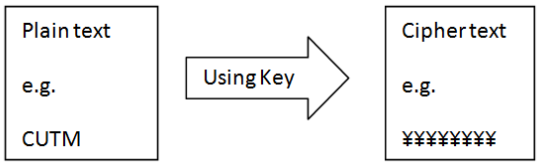

Fig. 1. Encryption process

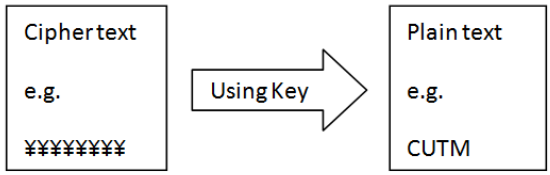

Fig. 2 Decryption process

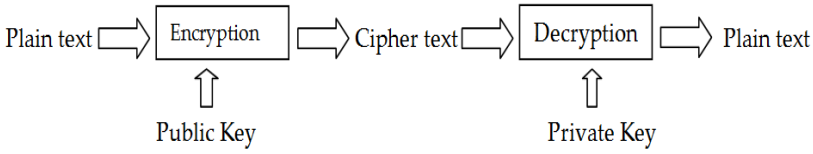

Fig. 3 Asymmetric key cryptography

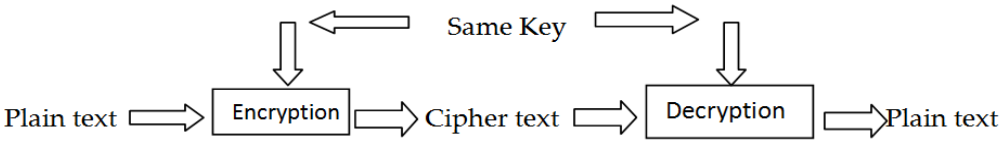

Fig. 4 Symmetric key cryptography

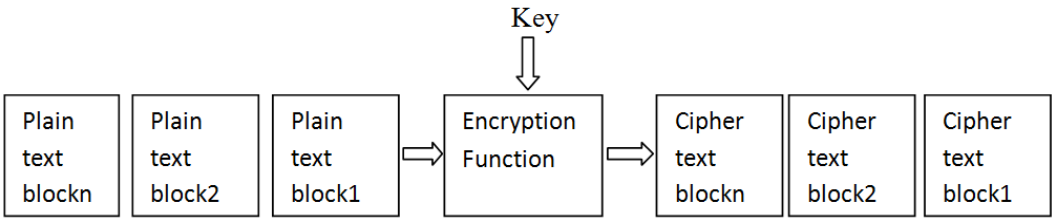

Fig. 5 Block Cipher

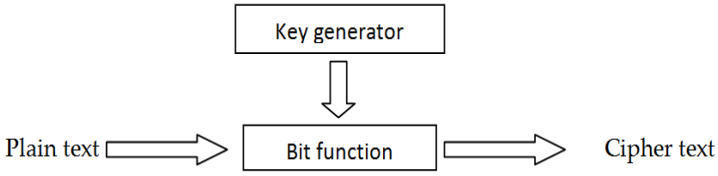

Fig. 6 Block Cipher

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REVIEW ARTICLE

A Review on the Antidiabetic Potential of *Murraya koenigii*

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ABSTRACT

Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycemia. Murraya Koenigii (M.K) belongs to the family Rutaceae (citrus family) commonly called "curry patta" in Hindi. The anti-diabetic activity is proved due to the presence of active constituents present in the plant. Certain active constituents present in the plant have the ability to treat many diseases like diabetes mellitus, inflammation, and vomiting and body pain. This review article mainly deals with the anti-diabetic activity of curry leaves. Phytoconstituents like flavonoids, terpenes, alkaloids, carbohydrates, niacin, alanine, cadinine, etc. are responsible for the hypoglycaemic effect in aqueous, methanolic and petroleum ether extracts. This study identified the plant part, the dose of the extract, standard drug and the best inducing chemical for diabetes by comparing the in-vivo and in-vitro studies. By comparing standard drugs like metformin, glibenclamide, and glimepiride, metformin was found to be highly effective. The standard drug metformin produced63.05% glucose reduction at a dose of 200mg/kg. Among aqueous, ethanolic and petroleum ether extracts, an aqueous extract shows good activity compared to the other two extracts. Hypoglycemic activity Different plant parts with different doses are used to identify which one has highly active. Aqueous leaf extract of Murraya Koenigii at a dose of 200mg/kg shows 85% glucose reduction occurs. It has more percentage of glucose reduction occurs compared with other plant parts and doses. Inducing chemical is STZ and alloxan; STZ is more potent than alloxan. STZ at a dose of 50mg/kg has the ability to induce diabetes.

Keywords: Diabetes Mellitus, Murraya Koenigii, Streptozotocin (STZ), Phytoconstituents

INTRODUCTION

Diabetes Mellitus is a group of metabolic disorders characterized by decreased insulin secretion or insulin action or both resulting in hyperglycemia [1,2]. Ancient time onwards, herbal preparations have been used for the treatment of many diseases including diabetes [3],due to their effectiveness, minimal side effects and low cost than synthetic drugs [1, 4].Over the various herbal substances available for the treatment of anti-diabetic study, Murraya Koenigii (M.K) is one among them used traditionally having a proven activity against Diabetes Mellitus. In Hindi, it is commonly called “curry patta”. In English, its leaves called "curry leaves". It is widely used as condiment and spice in India and other tropical countries [3, 4]. The main aim of this study is “to review the various invitro, invivo research works carried out on the anti-diabetic activity of M.K leaves and to identify its glucose reduction potential for future drug development. Certainactive constituents present in the M.K have a wide therapeutic activity for the treatment of vomiting, body pain and inflammation [5].

METHODOLOGY

Research and review articles published during the duration 2009 to 2019 were taken from different sources like Google Scholar, PubMed, and Research gate. Different plant parts were taken for the anti-diabetic study of the M.K by different researchers which had been reviewed here. The articles published in peer-reviewed journals were separated into in vitro and in vivo studies (Table 1 and Table 2) and then their antidiabetic potential is compared.

In-vivo antidiabetic Activity

Induction of Diabetes

Diabetes was induced by streptozotocin (STZ) and alloxan by various researchers in overnight fasted rats. To induce type II diabetes STZ was administered at a dose of 45mg/kg to 70mg/kg in Wistar and Sprague dawley rats.STZ is administered at a dose of 45mg/kgin adult male Wistar rats were divided into 5 groups (n=6),fasted initial glucose level was found to be 85.3±1.6, 265.0±1.3, 241.1±0.50, 226.2±0.47 and 215.1±1.67[1].Wistar rats administered STZ at a dose of 70mg/kg, animals were divided into seven groups (n=5). After 5 days of diabetes, induction fasted glucose level was found to be 94± 4.8, 340.6± 37.3, 259.2± 54.3, 231.4± 66.6, 261.6± 7.3, 218.4±45.4 and 203±15.7mg/dl[4].Fasted blood glucose level for STZ dose of 50mg/kg induced in 3 groups of male Wistar rats, after three days of induction initial glucose level was found to be 282.82±5.80, 418.56±10.62 and 387.75±11.37 [7]. Alloxan is administered at a dose of 120mg/kg i.p route in 5 groups (n=6) albino rats. After 72 hrs fasted Initial glucose level was found to be 84.6±1.8, 96.3±7.4, 91.5±5.6, 93.5±5.6 and 92.5±5.6[8]. Alloxan is administered at a dose of 35mg/kg induced in 5 groups of albino rats. Initial blood glucose level was found to be 86.59±10.21, 82.7±97.9483.8±9.73, 86.1±12.68 and 85.2±7.64 [9].Alloxan is administered at a dose of 150mg/kg p, o in 5 groups (n=6) having swiss albino mice. After 48 hrs fasting blood glucose level was found to be 89.76± 9.35, 310.2± 22.05, 311.12±27.35, 305.35±30.38 and 307.16 ± 30.38 respectively[10]. Alloxan is administered at a dose of 150mg/kg induced in seven groups (n=6) having albino Wistar rats. Initial BGL was found to be89.66±1.406, 273.66±1.838, 269.0 ±1.653, 271.0±1.612, 277.66±2.155, 271.83±1.447 and 269.33±1.820[11].For alloxan administered at a dose of 140mg/kg i.p induced in five groups (n=6) Wistar albino rats. After 72 hrs, the initial glucose level was found to be 82 ± 21, 276 ± 20, 215 ± 87, 215±87 and 177±4.9 [12].

Comparison of Various Extracts of M.K

Harneet Singh et al. conducted an anti-diabetic potential of aqueous and alcoholic root extract of M.Kin alloxan(150mg/kg) inducedalbino Wistar rats. Diabetes is induced by alloxan given by the intraperitonealroute. Aqueous root extract administered at a dose of 200mg/kg and 400mg/kg shows glucose reduction of 55.41% and

60.72% respectively. Whereas the alcoholic root extract of M.K administered on the same dose produced glucose reduction of 42.63% and 59.04% [11].400mg/kg dose of aqueous and alcoholic extract produces higher glucose reduction compared with 200mg/kg of the same extract. Whereas, at a dose of 200mg/kg of the aqueous extract is more active than alcoholic extract. But the difference may not be significant. From the above data, it may be concluded that no difference in the anti-diabetic activity of aqueous and alcoholic extract of M.K.

Dose-Response Relationship of M.K

Dinesh Kumar et al. studied the anti-diabetic activity in dry powder leaves of M.K extracted with petroleum ether at different doses 50mg/kg and 100mg/kg administered STZ (45mg/kg) in Wistar rats for 30 days.The treatment makes a glucose reduction of 31.76% and 37.72% respectively[1]. Another study conducted by Maha El Amin et al. with the same M.K at a dose of 100mg/kg and 200mg/kg in STZ (70mg/kg) induced male Wistar rats. The animals were treated for 13 days. At the end of the study percentage, glucose reduction was found to 57.93% and 62.64% respectively [4]. The treatment condition with 200 and 400mg/kg of M.K in Sprague dawley rats for 30 days produced a glucose reduction of 85% and 83% respectively reported by Imad M Al-Ani [2]. Based on the above literature various doses of M.K produces a significant reduction in glucose level in Wistar rats with respect to various doses of M.K. M.K is also found to be active in treating Sprague dawley rats but no significant difference in glucose level between the varying doses of M.K. The lower and higher dose produces similar glucose reduction in Sprague dawley rats treated with M.K. Petroleum ether extract of dry powder leaves of M.K at a dose of 50mg/kg and 100mg/kg(male wistar rats)shows a glucose reduction of 31.76% and 37.72% [1], whereasaqueous leaf extract 100mg/kg, 200mg/kg, 400mg/kg and 500mg/kg shows glucose reduction 57.93%[4],85% [2], 62.64% [4], 83% [2] and 47.74% [4]. There is no significant reduction between the various doses of M.K.

S. Yadhav et al. conducted an anti-diabetic study of powdered leaves of M.K at different concentrations such as 5%, 10% and 15% in alloxan (35mg/kg, i.p route) administered albino rats treated for 5 weeks. At the end of the study percentage, glucose reduction was found to be 3%, 1.76%, 1.49% respectively[9]. Another study was conducted by S.K Prasad et al. with the aqueous extract of M.K leaves at a dose of 250mg/kg and 500mg/kg in alloxan (120mg/kg, i.p route)induced albino rats. The animals treated for 21 days produced a glucose reduction of 0.44% and 0.22% respectively [8].M.C. Upadhye [12] from a study conducted proved anti-diabetic activity with the ethanolic extract of stem bark of M.Kat 125mg/kg and 250mg/kg in alloxan (140mg/kg, i.p) induced Wistar albino rats treated for 11 days. The percentage glucose reduction was found to be 28.42% and 41.05% respectively.Another study was conducted by Harneet Singhet al. with the aqueous and alcoholic root extract of M.K at 200mg/kg and 400mg/kg in alloxan (150mg/kg, i.p) administered albino Wistar rats. The animals were treated for 21 days. At the end of the study, glucose reduction was found to be 55.41% and 60.72% for aqueous extract and for alcoholic extract, it was found to be 42.63% and 59.04% respectively [11].Tembhurne.S.V et al.[10] administered 2.5ml/kg and 5ml/kg of aqueous fruit juice of M.K in alloxan (150mg/kg) induced Swiss albino mice for 15 days. The glucose reduction was found to be 46.08% and 53.24% respectively. The literatures that are discussed above indicate that various doses and different parts of M.K show a significant reduction in glucose level in Wistar rats. It was also observed that there was no significant difference in the glucose level when various doses of M.K was administered to Swiss albino mice.

Stem bark of M.K extracted with ethanol at 125mg/kg and 250mg/kg showed 28.42% and 41.05% glucose reduction respectively [12]. Alcoholic root extract of M.K at various doses 200mg/kg and 400mg/kg showed 42.63% and 59.04% glucose reduction[11].Powdered leaves of M.K at various concentrations such as 5%, 10% and 15% showed 3%, 1.76% and 1.49% glucose reduction[9].Aqueous leaf extract, fruit juice and root extract of M.K at various doses like 200mg/kg, 250mg/kg, 400mg/kg, 500mg/kg, 2.5ml/kg, 5ml/kg found to produce percentage glucose reduction of 55.41%[11], 0.44%[8]60.72%[11], 0.22%[8], 46.08% and 53.24% respectively [10].

Comparison of Standard

Dinesh Kumar et al. conducted an anti-diabetic study in Wistar rats using Glibenclamide as the standard drug. The percentage glucose reduction at a dose of 0.5mg/kg was found to be 44.84% [1].S.K Prasad et al.carried out hypoglycemic activity studies in adult albino rats. The standard drug used was Glibenclamide (3mg/kg) and the percentage glucose reduction was found to be 48.82% [6].Pratibha Chaturvedi et. al conducted an anti-diabetic study in male Wistar rats. Glibenclamide was used as the standard drug at a dose of 500mg/kg and the percentage glucose reduction was found to be 36.58% [7]. A hypoglycemic activity study was conducted by Maha El Amin et al.in male Wistar rats. Here metformin was used as the standard drug at a dose of 600mg/kg. It showed 59.66% glucose reduction[4] A study conducted by Harneet Singh et. al proved the anti-diabetic activity in albino Wistar rats using Metformin as the standard drug at a dose of 200mg/kg and glucose reduction was found to be 63.05% [11].The anti-diabetic potential of M.K was determined byM. C. Upadhye in Wistar albino rats. Metformin was the standard drug of choice at a dose of 50mg/kg and showed 47.36% glucose reduction[12].

Ahmed S.K et al. studied the anti-diabetic activity of M.K in albino rats. Here, Glimepiride is used as the standard drug at a dose of 2mg/kg and the percentage glucose reduction was found to be 0.07% [8].Anti-diabetic activity of fruit juice of M.K was proved by the Tembhurne S. V. et. al in swiss albino mice. Tolbutamide was used as the standard drug at a dose of 300mg/kg. The percentage of glucose reduction was found to be 56.06% [10]. From different researches conducted, % glucose reduction with the various doses of different synthetic standard drugs are compared (Table1). The standard drug Glibenclamide at various doses like 0.5mg/kg,3mg/kg and 500mg/kg showed 44.84%, 48.82% and 36.63 % glucose reduction respectively[1, 6, 7].In case of metformin, the doses selected are 50mg/kg, 200mg/kg and 600mg/kg and the percentage glucose reduction was found to be 47.36%, 63.05% and 59.66% respectively [12, 11, 6].When the standard drug glimepiride at a dose of 2mg/kg and tolbutamide at a dose of 300mg/kgwere used, the percentage glucose reduction was found to be 0.07% and 56.06% respectively[8, 10]. These observations show that standard drug metformin at a dose of 200mg/kg produces the highest glucose reduction of 63.05% than the other doses and produces better anti-diabetic activity compared to other standard drugs.

The anti-diabetic potential of M.K in comparison with synthetic drugs

The anti-diabetic activity of M.K, both aqueous and non-aqueous extracts (figure 1 and figure 2) were compared with different standard drugs.Dinesh Kumaret al. proved the anti-diabetic activity of M.K leaves in STZ induced adult male Wistar rats using Glibenclamide as standard drug. The percentage glucose reduction of petroleum ether extract of powdered leaves (50mg/kg and 150mg/kg) was compared with the Glibenclamide at a dose of 0.5mg/kg. Standard drug shows glucose reduction of 44.84%, which was found to be higher than the petroleum ether extract of 37.72% (150mg/kg) and 31.76% (50mg/kg) [1]. In an anti-diabetic study conducted by the Maha El Amin in STZ administered male Wistar rats, aqueous leaf extract at various doses (100mg/kg and 200mg/kg) and standard drug metformin (600mg/kg) were given.Metformin showed a higher glucose reduction of 59.66% when compared to aqueous extract of 57.93% and 62.64% [4].

Hypoglycemic activity of aqueous leaf extract of M.K was studied by the S.K. Prasad et al. in STZ induced adult albino rats. Aqueous leaf extract at a dose of 500mg/kg and standard drug Glibenclamide at a dose of 3mg/kg was administered. The result shows Glibenclamide has a greater activity of 48.82% when compared with the aqueous leaf extract 47.74% [6]. The slurry of dried powder leaves of M.K proved the anti-diabetic potential by a study conducted by Pratibha Chaturvedi et al. in STZ induced Male Wistar rats.In this study, an aqueous slurry of dried powdered leaves (250mg/kg) is compared with the standard drug Glibenclamide (500mg/kg).At the end of the study, standard drug Glibenclamide shows greater glucose reduction (36.58%) when compared to the aqueous slurry of dried powdered leaves 33.63% [7].

The anti-diabetic potential of aqueous leaf extract of M.Kwas proved by Ahmed SK et al. in alloxan-induced albino rats. Glimepiride is used as the standard drug at a dose of 2mg/kg compared with the two various doses of aqueous

extract. The results showed that Glimipiride at a dose of 2mg/kg resulted in only 0.07% and aqueous leaf extract resulted in 0.44% and 0.22% glucose reduction[8]. Tembhurne S. V.et al. proved the anti-diabetic activity of fruit juice of M.K in alloxan administered swiss albino mice. Here, Tolbutamide is used as the standard drug at a dose of 300mg/kg and compared with the aqueous fruit juice at various doses 2.5ml and 5ml. From the results, it was found that standard drug showed higher glucose reduction i.e., 53.24% when compared to the aqueous fruit juice [10]. Harneet Singhet al.determined the hypoglycemic effect of root extract of M.K in various doses (200mg/kg and 400mg/kg) in aqueous and alcoholic extract and compared with the standard drug metformin at a dose of 200mg/kg. At the end of the study, metformin showed 63.05% glucose reduced which was found to be higher than the plant two extracts [11].

M. C. Upadhyeet al. performed the anti-diabetic activity of M.K stem bark in Wistar albino rats and the diabetes-inducing chemical was alloxan.The standard drug used was metformin at a dose of 50mg/kg and glucose reduction was compared with the ethanolic extract of stem bark of M.K (125mg/kg and 250mg/kg). Metformin showed more reduction at a dose of 50mg/kg (47.36%) when compared to the extracts. Literatures show that for antidiabetic activitystudiesmostly used standard drugs are Glibenclamide and Metformin. In a study where the highest value of glucose reduction in M.K leaves[6]was obtained, percentage glucose reduction of aqueous extract, petroleum ether extract and an aqueous slurry of dried powdered leaves werecompared with the standard drug Glibenclamide. The study was carried out in adult albino rats and diabetes was induced by STZ at a dose of 50mg/kg for 15 days treatment period. The percentage glucose reduction was found to be 47.74% using aqueous extract of M.K leaves. Glibenclamide showed glucose reduction 48.82% at a dose of 3mg/kg,44.84% at0.5mg/kg and 36.58% at 500mg/kg [6, 1, 7].

In another study, antidiabetic activity of aqueous extract of M.K leaves (100mg/kg), aqueous and alcoholic root extract(200mg/kg and 400mg/kg), ethanolic stem extract (125mg/kg and 250mg/kg) and standard drug metformin (600mg/kg, 200mg/kg and 50mg/kg) were compared. The results indicates that aqueous leaf extracts show 57.93% glucose reduction in 100mg/kg,aqueous extract 62.64% (200mg/kg) and alcoholic root extract 42.63% (200mg/kg).The standard drug metformin at a dose of 200mg/kg (63.05%) shows highest glucose reduction than 600mg/kg (59.66%) and 50mg/kg (47.36%) [4,11,12].A significant glucose reduction occured when administered 200mg/kg (63.05) and 100mg/kg (57.93%) of aqueous leaf extract. But for 400mg/kg dose there was no increase in the %glucose reduction. Metformin shows greater glucose reduction in 200mg/kg (63.05%) compared to Glibenclamide.

Comparison of Anti-Diabetic Activity of Plant Parts With Doses

Leaves, roots, fruits and stem bark are the plant parts which are identified to have anti-diabetic potential[1,2,4,6,7,8,9,10,11,12]. AL-Ani et al. show thataqueous leaf extract leads to an 85%glucose reduction at 200mg/kg [2] and at 83% at 400mg/kg. As per El Amin et al., it was found to be 62.64% at 200mg/kg [4]. In another study, an aqueous and alcoholic root extract of M.K at 200mg/kg glucose reduction was found to be 55.41% and 42.63% [11] where the aqueous and alcoholic root extracts of M.K at a dose of 400mg/kg showed percentage glucose reduction of 60.72% and 59.04% respectively. The study conducted by El Amin et al. compared the activity of aqueous leaf extract and petroleum ether extract of dried leaf powder of M.K at a dose of 100mg/kg. The aqueous extract shows a greater percentage of glucose reduction (57.93%) than petroleum ether extract (37.72%). Aqueous fruit juice of M.K at a dose of 5ml shows 53.24% glucose reduced [10]. Another two studies indicated that aqueous leaf extract of 500mg/kg shows 47.74% [6]and 0.22% glucose reduction [8].

Tembhurne et al. found that aqueous extract of fruit juice at a dose of 2.5ml shows glucose reduction of 46.08% [10] Different studies conducted also proved that the ethanolic extract of stem bark at a dose of 250mg/kg shows 41.05% [12], an aqueous slurry of dried powder leaves shows 33.63% [7]and aqueous leaf extract shows 0.44% of glucose reduction occurs [8]. This indicates that the Ethanolic extract of stem bark has greater anti-diabetic potential than that of an aqueous slurry of dried powder leaves and aqueous leaf extract when administered 250 mg/kg. Few other

studies conducted also indicates that with petroleum ether extract of dry powder leaves at a dose of 50mg/kg showed 31.76% [1], ethanolic extract of stem bark at a dose of 125mg/kg showed 28.42% [12] and powdered leaves of M.K at different percentages (5%, 10%, 15%) shows 3%, 1.76% and 1.49% glucose reduction[9]. From these literatures, it is observed that aqueous extract of M.K leaves has greater anti-diabetic potential at 200mg/kg (85%) and lowest of0.22% at a dose of 500mg/kg when compared to other plant parts.

In-vitro Anti-Diabetic Activity

Dinesh Kumar et al. conducted an in-vitro anti-diabetic study of mahanimbine alkaloid. M.K leaves were extracted with petroleum ether and appreciable alpha-amylase inhibitory effects of 83.72±1.4µg/ml and alpha-glucosidase activity were found to be 99.89±1.2µg/ml[2].Inhibition of alpha-amylase and alpha-glucosidase limits postprandial glucose by reducing carbohydrate absorption.From this, we can assume that mahanimbine has the ability for the management of postprandial hyperglycemia. Sudha Ponnuswamy et al. proved the in-vitro human pancreatic amylase inhibitory activity by conducting porcine pancreatic alpha-amylase and human pancreatic amylase activity studies. Results show that isopropyl alcohol extract of M.K leaves shows ≥50% inhibition in Porcine pancreatic alpha-amylase (PPA) and 0.05µg/ml for Human pancreatic amylase (HPA) [13].From these, we can conclude that the inhibition of human pancreatic amylase activity, lead to the delay in the starch hydrolysis and thereby exhibit the hypoglycemic effect. The presence of an HPA inhibitor blocks the conversion of starch to glucose. Thus glucose formation is blocked in hyperglycemic conditions in human pancreatic amylase inhibitors. Mahesh Bhanudas et al. studied the alpha-amylase inhibitory potential of the ethanolic extract of M.K leaves. It shows 63.28% alpha-amylase inhibition [14].It delays the breakdown of carbohydrate thereby decreases glucose level.

Phytoconstituents Present in the Extract

Harish K Handra et al. proved the phytochemical Studies on M.K Leaves revealed the presence of constituents present in it. They showed the presence of carbohydrates, alkaloids, sterols, tannins, volatile oils, saponins, anthraquinone glycosides, and flavonoids. The fresh curry leaves contain terpenes, antioxidants and chlorophyll shows hypoglycemic activity and increased insulin secretion [15]. Vinuthan M. K et al. studied the effect of extracts of M.Kleaves on the levels of blood glucose and plasma insulin showed the hypoglycemic effects. The aqueous and methanolic extracts exhibited a significant anti-diabetic effect. The effect is due to the presence of alanine, leucine, carbohydrate, niacin, iron, and calcium in aqueous extract and the various constituents in the volatile oil like indole alkaloids such as mahanine and mahanimbine, sesquiterpene such as cadinene and monoterpene such as dipentene in methanolic extract of M.K[16].Phytoconstituents present in fresh curry leaves contain volatile oil and essential oils and also contain folic acid, carotene, beta carotene, chlorophyll, terpenes, and antioxidants exhibit hypoglycemic effect and increased insulin secretion [17]. R. Chaudary et al. carried out the variation in essential, trace and toxic elemental contents in M.Kand revealed the presence of the hypoglycemic effect. Several researchers conducted studies on the aqueous extract of curry leaves and showed the hypoglycemic effect without side effects and toxicity. The leaves are shown to reduce the blood glucose level where Cr, V, Mn, Zn, Cu are known to play an important role in the biological process especially in diabetes [18]. Irawan Wijaya Kusuma et al. proved the biological activity and phytochemical analysis of three Indonesian medicinal plants, M.K, Syzygiumpolyanthum, and Zingiber purpurea. Phytochemical analysis revealed the presence of carbohydrate, tannins, alkaloid, steroid, triterpenoid, and flavonoids present in the ethanolic extract of M.K leaves and twigs. Antidiabetic and immunomodulatory of the M.K were also proved [19].P. Arulselvan et al. proved the anti-diabetic effect of M.K leaves. The preliminary phytochemical screening of the ethanolic extract of M. koenigii showed the presence of biologically active ingredients such as alkaloids, flavonoids, glycosides, triterpenoids, phenols, etc. It is, therefore, conceivable that the biologically active ingredients present in the ethanolic extract of M. koenigii leaves exert their hypoglycemic effect by potentiating the beta-cells of the pancreas [20].

Manisha Vats et al. carried out the phytochemical screening and antimicrobial activity studies of roots of M.K (Linn.) and concluded that the phytochemicals are responsible for hypoglycemic activity. M.K is rich in carbazole alkaloids. The petroleum ether, chloroform, ethyl acetate and ethanol extracts of roots of the plant were used for the phytochemical analysis. The extracts were screened for the presence of different phytoconstituents like saponins, tannins, alkaloids, glycosides, steroids, and flavonoids. Phytochemical screening of petroleum ether and ethanolic extract of M.K roots showed the presence of sterols, carbohydrates, and flavonoids responsible for the anti-diabetic activity[21]. Ajay S et. al determined the phytochemicals present on M.K leaves. Curry leaves were found to have effective antioxidant, antidiabetic, antibacterial, antihypertensive and cytotoxic activities and also used in the treatment of bronchial respiratory difficulties. M.K leaves contain alkaloids like mahanine, koenine, koenigine, koenidine, girinimbiol, girinimibine, koenimbine, O-methyl murrayamine A, O-methyl mahanine, isomahanine, bismahanine, bispyrayafoline and other chemical constituents such as coumarin glycoside like scopotin, murrayanine, calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid. The volatile oil from leaves yielded diα-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpinol and caryophyllene.

From these M.K was able to produce antioxidant, antibacterial, antifungal, larvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and antihypertensive activities. Girinimbiol and Girinimbine, the most active carbazole alkaloids were isolated from the methanolic extract of M.K leaves and have shown to have effective hypoglycemic activity[22]. Shwetha Kesari conducted the studies on glycemic and lipidemic effects of M.K and showed that the aqueous leaf extract contains many active constituents include tannins, flavonoids, carbazole alkaloids. These are known to be bioactive for the management of diabetes. Certain alkaloids and flavonoids exhibit hypoglycemic activity and also for the regeneration of beta cells of the pancreas. Tannins are also shown to decrease the blood sugar level. Thus, the anti-diabetic activity of aqueous extract of M.K leaves may be due to the presence of more than one antihyperglycemic constituents or the synergistic activity of different constituents[23].

CONCLUSION

Different plant parts of M.K has been proved to have anti-diabetic activity. Studieson different extracts like petroleum ether, ethanolic extract, and aqueous extract show that aqueous extract produces greater anti-diabetic activity when compared to the ethanolic and petroleum ether extract and leaf extract was found to be highly active when compared with other plant parts. Aqueous extract of M.K leaves shows higher activity (85%) at a dose of 200mg/kg.Standarddrugs commonly used are metformin, glibenclamide, and glimepiride in different doses. Metformin at a dose of 200mg/kg shows 63.05% is found to be more active than other standards. In-vivo and in-vitro toxicity studies also have been conducted by researchers and M.Kwas found to be a very safe and promising plant drug for diabetes management.

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Table no: 1. The *In-vivo* Activity of M.K

Plant part	Animal/ Induction method	Treatment Duration	Extraction method	Dose	*In vivo* response		Reference
					Glucose level	% Reduction	
Leaves	Adult male Wistar rats,STZ 45mg/kg i.p	30 days	Petroleum ether extract of dry powder leaves	50mg/kg	217.0±0.61mg/dl	31.76%	Dinesh Kumaret al., 2010[1]
				100mg/kg	198.0±0.84mg/dl	37.72%	
			Glibenclamide	0.5 mg/kg	175.4±0.77mg/dl	44.84%	
			NA	DC	318.0±0.74mg/dl	-	
			NA	Control	92.2 ± 1.5 mg/dl	-	
Leaves	adult male Sprague dawley rats, 70 mg/kg STZ	30 days	Aqueous	200mg/kg	-	85%	Imad M Al-Aniet al. ,2017[2]
			Aqueous	400mg/kg	-	83%	
			Normal treated rats	M.K 400 mg/kg	-	-	
			NA	DC	-	-	
			NA	Control	-	-	
Leaves	Male Wistar rats,70mg/kg STZ	13 days	Metformin	600mg/kg	97.6± 4.0mg/dl	59.66%	Maha El Aminet al. ,2013[4]
			NA	DC	242.0± 36.2mg/dl	-	
			NA	Control	94.0± 5.1mg/dl	-	
			Aqueous	100mg/kg	101.8± 21mg/dl	57.93	
			Aqueous	200mg/kg	90.4± 10.3mg/dl	62.64%	
Leaves	Adult albino rats, 50mg/kg STZ	15 days	Aqueous	500mg/kg	-	47.74%	S.K. Prasadet al. ,2009[6]
			Glibenclamide	3mg/kg	-	48.82%	
			NA	Control	-	-	
			NA	DC	-	-	
The slurry of dried powder leaves	Male Wistar rats,STZ 50 mg/kg i.p	24 hours	The aqueous slurry of dried powdered leaves	250mg/kg	403.23±11.37mg/dl	33.63%	Pratibha Chaturvediet al. ,2014[7]
			NA	DC	301.74± 5.80mg/dl	-	
			Glibenclamide	500mg/kg	412.12±10.6237mg/dl	36.58%	
Leaves	Albino rats, Alloxan 120mg/kg i.p	21 days	Aqueous	250mg/kg	271.5±2.6mg/dl	0.44%	Ahmed SKet al., 2017[8]
			Aqueous	500mg/kg	273.3±3.2mg/dl	0.22%	
			Glimiperide	2mg/kg	272.5±2.6mg/dl	0.07%	
			NA	DC	272.7±3.9mg/dl	-	
			NA	Control	82.83.7±2.1 mg/dl	-	
Leaves	Albino rats, alloxan 35mg/kg i.p	5 weeks	Powdered leaves	5%	199.59±15.83 mg/dl	3%	S. Yadavet al., 2002[9]
				10%	197±15.50 mg/dl	1.76%	
				15%	196.5±16.93	1.49%	
			NA	DC	193.6±12.7 mg/dl	-	
			NA	Control	214.7±12.11 mg/dl	-	
Fruit juice	Swiss albino mice,alloxan 150mg/kg p.o	15 days		2.5ml/kg	210.44±33.19mg/dl	46.08%	Tembhurne S. Vet al., 2009[10]
				5 ml/kg	182.52±24.165mg/dl	53.24%	
				300mg/kg	179.32± 22.10 mg/dl	56.06%	
				Control	89.58± 7.06 mg/dl	-	
				DC	390.34± 27.59 mg/dl	-	

Root extract	Albino Wistar rats,alloxan 150mg/kg i.p	21 days	Metformin	200mg/kg	110.33±2.431mmol/l	63.05%	Harneet Singhet al.,2012[11]
			Aqueous	200mg/kg	133.17±1.851mmol/1	55.41%	
			Aqueous	400mg/kg	117.3±1.978mmol/1	60.72%	
			Alcoholic	200mg/kg	171.33±3.756mmol/1	42.63%	
			Alcoholic	400mg/kg	122.33±1.585mmol/1	59.04%	
Stem Bark	Wistar albino rats,alloxan 140mg/kg i.p	11 days	Ethanolic extract of stem bark	125mg/kg	136 ± 4.8mg/dl	28.42%	M.C. Upadhyeet al.,2019[12]
				250mg/kg	112 ± 19mg/dl	41.05%	
			Metformin	50mg/kg	100 ± 5.1mg/dl	47.36%	
			NA	Control	92 ± 0.3mg/dl	-	
			NA	DC	190 ± 5.3mg/dl	-	

Table No: 2. *In-vitro* Activity of M.K

Sl No	*In vitro* Assay	Result			References
		Petroleum ether	Isopropyl alcohol	Ethanolic	
1	Alpha-Amylase inhibitory activity	83.72±1.4µg/ml			Dinesh Kumaret al., 2017[2]
	Alpha Glucosidase inhibitory activity	99.89±1.2µg/ml			
2	Porcine pancreatic alpha-amylase (PPA)		≥50%		Sudha Ponnuswamyet al.,2011[13]
	Human pancreatic amylase (HPA)		0.05 µg/ml		
3	Alpha-amylase inhibitory activity			63.28%	Mahesh Bhanudaset al., 2012[14]

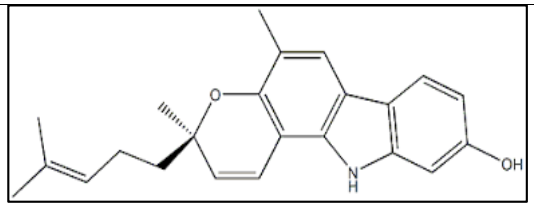

Fig.1.Mahanine

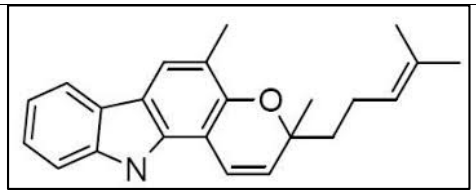

Fig.2.Mahanimbine

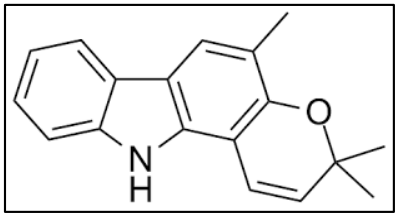

Fig.3.Girinimbine

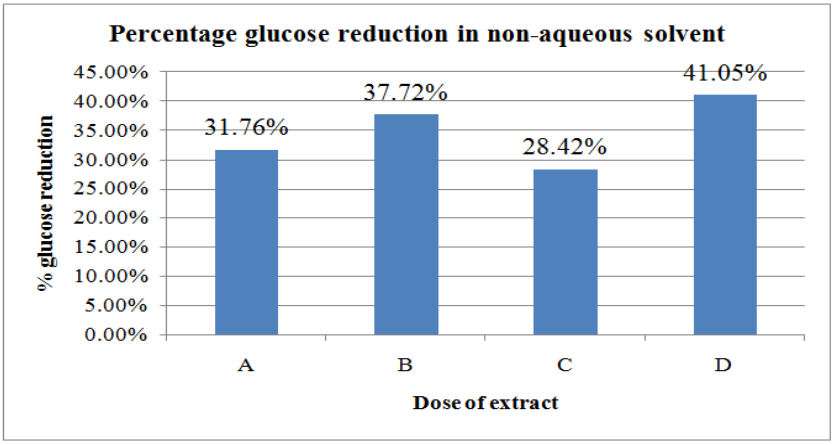

Fig. 4: Percentage of blood glucose reduction in non-aqueous extract

A: Petroleum extract of powdered leaves 50mg/kg, B: Petroleum extract of powdered leaves 100mg/kg, C: Ethanolic extract of stem bark 125mg/kg, D: Ethanolic extract of stem bark 250mg/kg

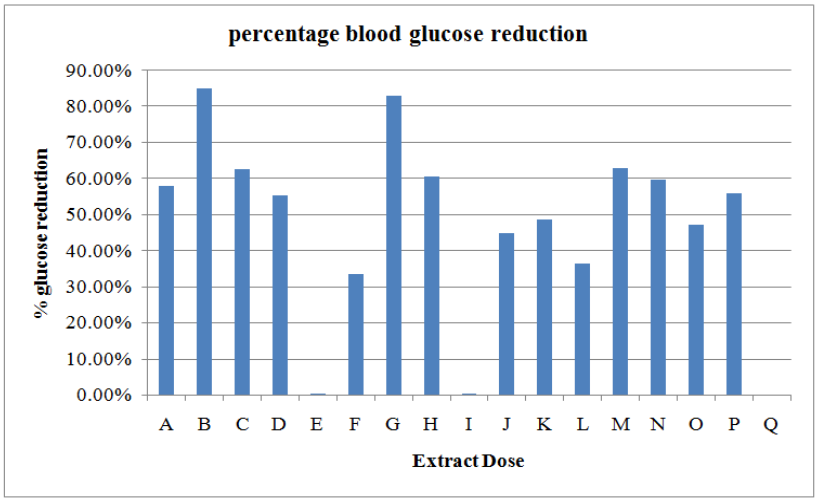

Fig. 5: Percentage of blood glucose reduction

A: Aqueous extract of powdered leaves 100mg/kg, B: Aqueous extract of powdered leaves 200mg/kg, C: Aqueous extract of powdered leaves 200mg/kg, D: Aqueous extract of powdered leaves 200mg/kg, E: Aqueous extract of powdered leaves 250mg/kg, F: Aqueous extract of powdered leaves 250mg/kg, G: Aqueous extract of powdered leaves 400mg/kg, H: Aqueous extract of powdered leaves 400mg/kg, I: Aqueous extract of powdered leaves 500mg/kg, J: Standard drug Glibenclamide 0.5 mg/kg, K: Standard drug Glibenclamide 3mg/kg, L: Standard drug Glibenclamide 500mg/kg, M: Standard drug Metformin 200mg/kg, N: Standard drug Metformin 600mg/kg, O: Standard drug Metformin 50mg/kg, P: Standard drug Tolbutamide 300mg/kg, Q: Standard drug Glimepiride 2mg/kg.