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RESEARCH ARTICLE

An *In-silico* Investigation of Phytochemicals from *Brassica juncea* (Mustard) as Antibacterial Agent against Diarrhoea

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ABSTRACT

Phytochemicals are considered to benon-nutritive compounds obtained from plants. Several investigations have reported that plant extracts can be usedin curing Diarrhoea, as they contain several phytochemicals. Diarrhoea is a common disease, which is caused by *Escherichia coli.* One of the key enzymes known to be involved in its biochemical pathway for the biosynthesis of aromatic amino acidsis Shikimate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was validated through BIOVIA using Discovery Studio. The strength of the interaction was evaluated based on -CDOCKER energy and -CDOCKER interaction energy. Higher positive values during evaluation using both the parameters indicated that, out of the selected phytochemicals Kaempferol and Quercetinare effective in deactivatingthe Shikimate dehydrogenase enzyme, thereby interrupting the life cycle of *Escherichia coli*.

Key words: Phytochemical, BIOVIA, Discovery studio, *Brassica juncea*, *Escherichia coli*

INTRODUCTION

The use of natural products including medicinal plantswith therapeutic properties is as ancient as is the human civilization, and for centuries natural products have been considered asthe main source of drugs. About 25% of the drugs that are prescribed worldwide comes from plants, with more than 121 such compounds still currently in use.

At the beginning of the 21st century, 11% of the identified 252 drugs were considered basic and essential by the WHO and were exclusively reported to have origin from flowering plants. In recent years, with the development of technology, there has been serious rethinking on study of medicinal plants and traditional medicinesas the source for new bioactive metabolite production, for the treatment of various ailments [1, 2, 3, 4]. This is primarily due to the steady progress in drug development from synthetic perspective, and the affirmationby both developed and developing nations towards the herbal remedies. Traditional medicinal practices arean integral part of alternative medicine. However, their efficacy and mechanism of action have not been thoroughly studiedby the scientific community in most cases. These single or polyherbal preparations often produce beneficial responses to several diseases in response to the active chemical constituents present in them [5].

Epidemiological studies have revealed that, reduced risk of developing chronic diseasesis strongly associated with the high dietary intake of fruits, vegetables and whole grains. Dietary supplements, nutraceuticals and functional foods have succeeded in gaining attention owing to their impact on human health. Phytochemicals are bioactive non-nutrient plant based compounds in fruits, vegetables, grains, and other plant foods that reportedto the risk of major chronic diseases. Phytochemical extracts from plants have greater antioxidant and antiproliferative activities and it is further enhanced by the combination of various phytochemicals. The additive and synergistic effects of such phytochemicals in the dietare responsible for such increased antioxidant and anticancer activities. This explains the enhanced activity of combination of natural phytochemicals over a single phytochemical. This suggests that antioxidants are best acquired through whole food consumption, rather than expensive dietary supplements [6].

The medicinal values ofplants lie in their chemical active substances which shows a definite physiological action on the human body. The most important of the bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds [7]. In the recent years, infections have kept increasing to a greater extent due to which antibiotics resistance effects have become a significant therapeutic problem [8]. Natural products from higher plants could possibly pose new resources with novel mechanism of action on many relevant diseases [9, 10]. such compounds could be more effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects associated with synthetic antimicrobial components [11].

Brassica juncea Czern. and Coss., also commonly known as Indian mustard, Chinese mustard, oriental mustard, mustard green or leaf mustard, is a species belonging to the mustard family of Brassicaceae (cruciferous) plants [12]. *B. juncea* is an economically important plant in India and is majorly used in recipes. Several parts of the plant are edible and are used in a range of folk medicines and spices. The mustard seeds are traditionally used for the treatment of some diseases, such as, muscular rheumatism, inflammatory neuralgic affections, dengue and vomiting [13]. Several pandemics of cholera have occurred worldwide in the past, there is a general disposition to diarrhoea or to painful affections of the bowels in those places where Asiatic cholera was prevalent. The use of mustard hip bath during diarrhea has been reported to have significant relieving effects during the early period [14]. Enterotoxigenic *E. coli* is one of the most common cause of Diarrhoea [15]. Hence, the current study focuses on the identification of the phytochemical of *Brassica juncea* that could be responsible to cure Diarrhoea caused by *Escherichia coli.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systems of France) was used for the data analysis. This software utilizes machine learning techniques for predictingthe level of molecular interactions.

List of Phytochemicals

Phytochemicals are known to beproduced by plants as secondary metabolites in orderto protect them from predators. The potential threats to plants could include bacteria, viruses, fungi, etc. When these plants or their parts are consumed by humans these phytochemicals fight against the threats to human health. These phytochemicals have prospectiveas poisons as wellas traditional medicine. It has already been established that *Brassica juncea* plant belonging to Brassicaceae family has potential to help controlling Diarrhoea. Several published works have reported *Brassica juncea* to contain kaempferol, quercetin, limonene, neoeriocitrin, vetiselinenol, etc. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling Diarrhoea.

Enzyme present in *Escherichia coli*

It has been reported that Diarrhoea can be caused as a result of *Escherichia coli* infestation. Several metabolic cycles in the bacteria are responsible for its life cycle and survival. The screening for various enzymes involved in the metabolism of *E. coli* was carried out using BRENDA enzyme database. It has been found that Shikimate dehydrogenase enzyme (protein database code 1 NYT) is involved in some vital metabolic process and are very crucial for survival of this bacteria.

Molecular docking

Molecular docking methods are useful in identifying the phytochemical from plant extracts, that can act as 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 interactions and perform molecular docking. During the process, the Spatial data files (sdf) files for the phytochemicals that are reported from *Brassica juncea* plant were downloaded from the website. The protein database code of the Shikimate dehydrogenase enzyme was identified. The active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was carried outusing the CDocker protocol of BIOVIA under “receptor-ligand interaction”. The enzyme molecule was treated as the receptor molecule and the phytochemical as the ligand. The“-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. The high positive values of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the essential phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Figure 1 shows the active site of the Shikimate dehydrogenase enzyme. It appears as light green in 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 shows that Shikimate dehydrogenase-Kaempferol interaction has the highest positive value of -CDOCKER energy (19.28) and minimum value of the difference (8.44) between -CDOCKER interaction energy and -CDOCKER energy followed by Quercetin. Thus the results indicated that Kaempferol and Quercetin can effectively deactivate the Shikimate dehydrogenase enzyme there by interrupting the biological cycle of *Escherichia coli*. Higher positive values for Kaempferol indicated that it was the most active ingredient against *Escherichia coli*. Alpha-linolenic, piperine acid and Camphor cannot interact

with Shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing Diarrhoea caused by *Escherichia coli* are Kaempferol and Quercetin.

CONCLUSION

It was previously known that *Brassica juncea* plant has medicinal action against Diarrhoea, which is caused by *Escherichia coli*. This study was carried out to provide the experimental evidence of this observation. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical (Kaempferol, Quercetin, Alpha-linolenic acid, Piperine, Camphor), which can have a significant interaction with the vital enzyme (Shikimate dehydrogenase) of the microbe. It was found that Kaempferol and Quercetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the bacteria. Alpha-linolenic acid, piperine and Camphor cannot deactivate the enzyme. Thus, this study explains that the presence of Kaempferol and Quercetin provides medicinal values to *Brassica juncea* against Diarrhoea caused by *Escherichia coli*.

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Table 1. Results of CDocking of phytochemicals with Shikimate dehydrogenase enzyme

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	Difference between -CDOCKER interaction energy and -CDOCKER energy
1	Alpha-linolenic acid	-12.52	33.75	46.27
2	Quercetin	23.97	34.57	10.6
3	Camphor	-128.06	-35.85	92.21
4	Kaempferol	19.28	27.72	8.44
5	Piperine	-6.59	30.96	37.55

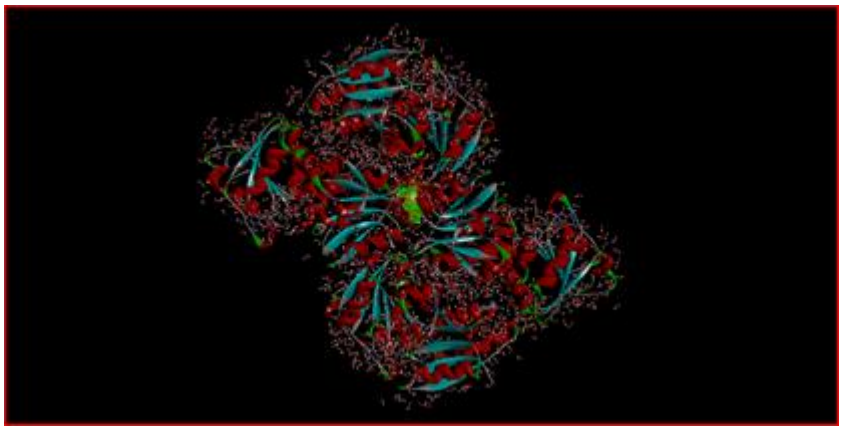

Figure 1. Active site of Shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In-silico Molecular Docking Studies and Activity of Anthraquinone in *Chrysopogon zizanioides* (Vetiver) Against Sepsis Caused by *Staphylococcus aureus*

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ABSTRACT

Phytochemicals are non-nutritive chemicals which are obtained from plants. It has been reported that plant extract has use as acure for sepsis. Plant extracts are reported to contain various phytochemicals in them, that are beneficially is treatment of several infections. Sepsis is caused by *Staphylococcus aureus.* One of the key enzymes involved in its biochemical pathway is Lactate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using BIOVIA Discovery Studio. The strength of the interaction between the molecules was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Anthraquinone can effectively deactivate the Lactate dehydrogenase there by interrupting the life cycle of *Staphylococcus aureus*.

Key words: Phytochemical, BIOVIA, Discovery studio, *Chrysopogon zizanioides, Staphylococcus aureus*

INTRODUCTION

Plant constituents still remain as an important resource to combat serious illness in the current era, irrespective of the massive development in synthetic drug production. The traditional medicinal methods, particularly the use of medicinal plants, play a vital role to cover the basic health requirements in the developing nations. The medicinal

value of these plants owes to the chemical active substances that promotes a definite metabolic action on human body. With in the recent past, infections have increased to a large extent and antibiotics resistant activities have become an ever-increasing therapeutic problem [1]. Natural products higher plants could set off to be the impending source of antimicrobial agents with possibly novel mechanisms of action [2, 3], as they are effective in the treatment of infectious diseases and can simultaneously mitigate many of the side effects which are associated with synthetic antimicrobials [4]. Plants have illimitable resources to synthesize aromatic substances, mainly secondary metabolites, of which more than 12,000 metabolites have been isolated, which still counts to beless than 10% of the total [5]. These aromatic substances are used by plants as defensive molecules against predator microorganisms, insects and herbivores [6]**.** A medicinal plant is any plant which contains a metabolite that canbe used for therapeutic applications or which contains substances that that have use asprecursors for the synthesis of useful drugs [7].

Vetiver or khus (*Vetiveria zizanioides*), a tall, perennial, miraculous grass, a native of India was first developed for soil and water conservation by the World Bank in themid-1980s. It produces spongy, highly branched, root system (khus roots) with fine rootlets, containing fragrant oil, having application in the form of perfume [8]. It is also a source of vetiver oil, having use as a fixative in blending cosmetics, in fragrance of incense sticks, soaps, soft drinks, pan masala. It also has applications in medical sector such as, strengthening of bones, treatment of rheumatism, gout, arthritis, muscle aches, dryness, cramps and dry skin [9]. Various parts of this multipotent medicinal planthave traditionally been used by the Indian tribes communities for treating various ailments, diseases and disorders including boils, burns, toothache, weakness, headache, fever, malarial fever, urinary tract infection, epilepsy, rheumatismand as an anti-helminthic [10, 11].

The extracts of *Vetiveria zizanioides* are profoundly explored for their use in Ayurvedic medicine. The metabolic constituents present in the plantare Vetiverol, Vetivone, Khusimone, Khusimol, Vetivene, Khositone,Terpenes, Benzoic acid, Tripene-4-ol, s-Humulene, Epizizianal,Vetivenylvetivenate, Iso khusimol, S-vetivone, Vetivazulene. Ayurvedic literature has cited the plant to be useful in digestive, carminative stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, antigout, anthelmintic, antimicrobial and diuretic [12]. *Staphylococcus aureus* is the leading cause of several nosocomial and community-associated infections, such asmild superficial skin infections, severe sepsis, bacteremia, osteomyelitis, implant-associated heart valve and native valve endocarditis. Antibiotic resistance has turned out to be a major concern worldwide, specially for the treatment of infection caused by *S. aureus*. Drug tolerant species are identified to be significant contributors in chronic persistent infections and recurrent infections. Clinically, infections reported from*S. aureus* such as, soft tissue infections, osteomyelitis, prosthetic joint infections, endocarditis and infection caused due to biofilm formation, on indwelling device is difficult to cure with the present antibiotics. This is because the antibiotics currently available are mainly active against actively growing bacteria but show poor outcome against the non-growing persisters. However, some essential oils are found to be active against *S. aureus* such as, Tea tree, Cajeput, Glove bud, Lavender, Sleep tight, Vetiver, Palo santo, Sage oil, Yarrow [13]. The current study focuses on the identification of the phytochemical of *Chrysopogon zizanioides* responsible to cure sepsis caused by *Staphylococcus aureus.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used for analysis. This software is enabled with machine learning techniques for predictingthe level of molecular interaction

List of Phytochemicals

Phytochemicals are components produced by plants as secondary metabolites to protect themselves from predators. Most of the potential threats to plants includes microorganisms such as bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans the phytochemicals constituents that gets ingested, fights of threats to human health. Some phytochemicals could be categorized as poisons and others as traditional medicine. Works reported till date suggests that *Chrysopogon zizanioides* contains Alpha-vetivones, Anthraquinone, Beta-vetivones, Cloven and vetiselinenol, etc.as useful metabolites. It has already been established that *Chrysopogon zizanioides* plant belonging to Poaceae family has potential to help controlling sepsis. Hence, the current study is focused on identification of the particular phytochemical responsible for inhibiting and controlling sepsis.

Enzyme found in *Staphylococcus aureus*

It has been reported that sepsis can be caused as a result of severe infection due to *Staphylococcus aureus*. Various metabolic processoccurs in bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. BRENDA enzyme database was used for identification and enlisting different enzymes found in *Staphylococcus aureus*. It has been found that Lactate dehydrogenase (protein database code 3D4P) is actively involved in some metabolic pathways and isvery crucial for the survival of this particular microbe.

Molecular docking

Molecular docking methods has been used for identifying the phytochemical from the selected plant, that could act as a ligand to form strong covalent bond with the bacterial protein for successfully inhibition of the microbe. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and to perform molecular docking. In this process first the SDFfiles for the phytochemicals found in *Chrysopogon zizanioides* plant were downloaded from the website. The protein database code of the Lactate dehydrogenase was identified from the website. The active site of the enzyme was identified via “receptor cavity” protocol under "receptor-ligand interaction" menu. Molecular docking was done using 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. During the study“-CDOCKER_ENERGY” and “-CDOCKER_INTERACTION_ENERGY” were used as indicator for the quality of molecular docking. Those with high positive value indicators presented a good interaction between the ligand and the receptor. Hence, the interactions showing high values could indicate the major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Figure 1 shows the active site of the Lactate 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 that is 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 interactions that exists between a protein and a ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) minor differences in-CDOCKER energy and -CDOCKER interaction energy [14]. Table 1 shows that Lactate dehydrogenase-Anthraquinone interaction has the highest positive value of -CDOCKER energy (21.51) and minimum value of the difference (5.37) between - C DOCKER interaction energy. Thus, the results indicated that Anthraquinone can effectively deactivate Lactate dehydrogenase thereby interrupting the biological cycle of *Staphylococcus aureus*. Higher positive values for Anthraquinone indicated that it was the most active ingredient against *Staphylococcus aureus*. Alpha-vetivones, Beta-

vetivones, Cloven and vetiselinenol could not interact with Lactate dehydrogenase. Thus, the key phytochemicals preventing sepsis caused by *Staphylococcus aureus* is Anthraquinone.

CONCLUSION

It has been previously reported that *Chrysopogon zizanioides* has medicinal action against sepsis. Sepsis is caused due to severe infection 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 (Alpha-vetivones, Anthraquinone,Beta-vetivones, Cloven and vetiselinenol), which can have a significant interaction with the vital enzyme (Lactate dehydrogenase) of the microbe. It was found that Anthraquinone can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha-vetivones, Beta-vetivones, Cloven and vetiselinenol could not deactivate the enzyme. Thus, this study could explain that the presence of Anthraquinone provides the medicinal values to *Chrysopogon zizanioides* against sepsis caused by *Staphylococcus aureus*.

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Table 1. Results of CDocking of phytochemicals with Lactate dehydrogenase enzyme

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Alpha-vetivones	-20.58	33.13	53.71
2	Anthraquinone	21.51	26.88	5.37
3	Beta-vetivones	-31.96	31.90	63.86
4	Cloven	-21.05	28.82	49.87
5	vetiselinenol	-13.41	32.22	45.63

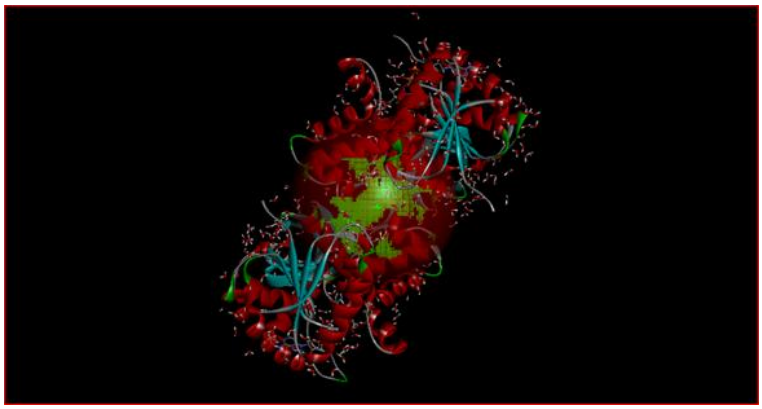

Figure 1. Active site of Lactate dehydrogenase enzyme

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RESEARCH ARTICLE

Evaluation of Phytochemical Compounds from *Curcuma longa* (Turmeric) as a Potential Drug against Sinusitis: An *In-silico* Approach

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ABSTRACT

Phytochemicals are bioactive and non-nutritive compounds that are obtained from plants. It has been reported that plant extract from *Curcuma longa* can be used to cure Sinusitis. The plant extract contains a list of phytochemicals that have shown tremendous beneficial effects.They ha ve a huge impact on the health care system and may provide medical related health benefits including the prevention and treatment of diseases and some physiological disorders. Sinusitis is caused by *Streptococcus pneumonia*, one of the key enzymes involved in its biochemical pathway is ribitol-5-phosphate 2-dehydrogenase (NADP+) enzyme. The molecular docking of the phytochemicals present in *Curcuma longa* was carried out with the enzyme using BIOVIA Discovery Studio. The strength of the interaction and efficacy was evaluated based on -CDocker energy and -CDocker interaction energy. Values skewed towards the positive side for both CDocker energy and -CDocker interaction energy indicated that out of different Phytochemicals Demethoxycurcumin and flavinoids can effectively deactivate the ribitol-5-phosphate 2-dehydrogenase (NADP+) enzyme thereby interrupting the life cycle of *Streptococcus pneumoniae*.

Key words: Phytochemical, BIOVIA, Discovery studio, *Curcuma longa, Streptococcus*

INTRODUCTION

In modern times, life has become 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 common health hazards such as blood pressure, diabetes, obesity. A little attention if paid towards the changing lifestyle could prevent such lifestyle related diseases from increasing rapidly. Along with coordination of lifestyle, several herbal remedies and heel the aliments that are caused during the lifetime of human beings. For this reason, several scientists have initiated exploring the phytochemicals present in plants for their health benefits. The "phyto-" of the word phytochemicals is derived fromthe Greek word *phyto*, as it is originating from plants. Hence, phytochemicals are plant-based chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds that are mostly found in fruits, vegetables, grains, and other plant foods that have been linked to decrease in the risk of major chronic diseases [1].

Medicinal plants have a therapeutic importance to the health of individuals and communities. The importance of these plants lies in some chemical components that produce a definite physiological action on the human health. The most important of these bioactive substances are alkaloids, tannins, flavonoids, and phenolic compounds [2]. The plant-based conventional medicine systems continue to play an important role in the health care system. The demand of herbal based medicine, health products, pharmaceuticals, food supplements, nutraceuticals, cosmetics are gradually increasing worldwide. In the current era, the natural products represent more than 50% of all drugs that have come into pharmaceutical use. More than 50% the approved herbal drugs during the last few decades are either directly or indirectly from naturalresources, including plants, microorganisms, fungi and animals [3]. Phytochemicals with nutraceutical properties present in food items plays a significant role in improving the health quality of human life. This is due to their beneficial impact on human health as they can provide protection against numerous diseases or disorders such as cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers, osteoporosis and associated disorders [4].

A number of diseases are caused by pathogenic micro-organism due to their invasive property and toxigenesis. Synthetic antibiotics which are used against such pathogens can cause oxidative stress leading to damage in the genetic material in human cells, but this effect can bealtered by antioxidants. Thus, as an alternative, medicinal plants can be used as they contain phytochemicals and antioxidants that can pave a way for development of antimicrobial drug along with having applications in cancer therapy.

Medicinal herbs represent an important source of antimicrobial agents and are widely used these days, either directly as folk remedies or indirectly in the preparation of synthetic pharmaceutical. World Health Organization (WHO) quoted that more than 80% of the world's population depends on traditional herbal medicine for primary healthcare. Since ages various ailments and infectious diseases have been known to be treated using herbal remedies throughout the world. Thus, researches are increasingly turning their attention to natural products, either as pure compounds or as standardized plant product, for new leads to develop advanced drugs against microbial infections. Turmeric (*Curcuma longa*) belongs to family Zingiberaceae. It is a perennial herb and is mostly used in countries like India, Pakistan and China.It is Cultivated in tropical and subtropical area of the earth. It is popularly known as Haldi in Pakistan and India. Since ancient times it is mostly used as a house hold therapy to manage several physiological ailments. The particular aroma of turmeric rhizome is due to the aromatic volatile oil like turmer one. Currently India is the biggest producer and consumer of turmeric in many aspects. China comes second as a producer of different spices including *Curcuma longa* and is followed by several other subcontinent countriesin the world [5]. *C. longa* contains essential oil, alkaloid, starch grains, yellow matter curcumin and other curcuminoids, turmeric oil, caproic acid as a free acid and valeric acid as a combined acid. Distillation of oil yields 2% d-sabinene, 1% α-phellandrene and 3% cineole from the lower fraction. The middle fraction yields 30.5% zingiberene and the higher

fraction shows a mixture of sesquiterpene hydrocarbons and sesquiterpene alcohol (50.5%). The oil contains small amounts of sesquiterpenes, α- and β-pinene, camphor, camphene and curcumenes. Turmeric contains an essential oil that is, zingiberene. Examination of its chemical composition has reported it to contain certain proteins, carbohydrates and fiber also [6]. It is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice, a dietary pigment. It is also used as Indian folk medicine for the treatment of various diseases. It also finds application in the textile and pharmaceutical industries, and in Hindu religious ceremonies in several forms. Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis [7]. Pathogen most commonly associated with acute sinusitis, includes *Streptococcus pneumoniae* [8].*S. pneumoniae* is a gram-positive coccus and is a member of the lactic acid bacteria. Bacteria belonging to the genus Streptococcus live in association with animal hosts, as either pathogenic or commensal organisms [9]. This study focuses on the identification of the phytochemical of *Curcuma longa* responsible to cure Sinusitis caused by *Streptococcus pneumoniae.*

MATERIALS AND METHODS

Software used

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

List of Phytochemicals

Phytochemicals are produced by plants as secondary metabolites mainly to protect them from predators. These plants or their parts when consumed by humans help fight off threats to human health. Some phytochemicals have been used as poisons and others as traditional medicine. Published works have reported *Curcuma longa* to contain Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin etc. It has already been established that *Curcuma longa* plant belonging to family Zingiberaceae has potential in controlling Sinusitis. The current study is focused on identification of the particular phytochemical responsible for inhibiting and controlling Sinusitis.

Enzyme found in *Streptococcus pneumoniae*

It has been reported that Sinusitis can be caused as a result of Streptococcal infestation. Various metabolic cycles in bacterial life cycle are required for their survival. These metabolic cycles require various enzymes in order to regulate them. BRENDA enzyme database was used to identify and screen various enzymes found in *Streptococcus pneumoniae*. It has been found that ribitol-5-phosphate2-dehydrogenase (NADP+) enzyme (protein database code 2VSI) is involved in some metabolic and crucial activities required for the survival of the particular microorganism.

Molecular docking

Molecular docking method has been used to identify the phytochemicals from *Curcuma longa*, that could act as ligand and form strong covalent bond with the bacterial protein to successfully inhibit it. The Discovery studio module of BIOVIA software was used for identifying molecular interaction and to perform molecular docking. During the process, the sdf files for the phytochemicals found in the *Curcuma longa* plant were downloaded. The protein database code of ribitol-5-phosphate 2-dehydrogenase enzyme was identified. The active site of the enzyme was identified via “receptor cavity” protocol under "receptor-ligand interaction" menu. Molecular docking was carried out using the CDocker protocol of BIOVIA software under “receptor-ligand interaction”. The enzyme molecule was

considered to be the receptor molecule and the phytochemical as the ligand molecule. The"-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. High positive value during the docking studies were indicator for 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

Figure 1 shows the active site of the enzyme ribitol-5-phosphate 2-dehydrogenase. It appears as light greenin color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm, which is grid-based molecular docking technique and is optimized for accuracy as well. 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 most efficient interaction was chosen based on the positive value of -CDOCKER energy and small difference between -CDOCKER energy and -CDOCKER interaction energy [10].Table 1 shows that ribitol-5-phosphate 2-dehydrogenase (NADP+) interaction with bis-demethoxycurcumin has the highest positive value of -CDOCKER energy i.e. 37.27and a minimum value in the difference i.e. 5.66 between - C DOCKER interaction energy and - C DOCKER energy followed byphlobatannins, Demethoxycurcumin, tannis and flavinoids. Thus, the results indicated thatbisdemethoxucurcumin, phlobatannis,Demethoxycurcumin, tannis and flavinoids can effectively deactivate the ribitol-5-phosphate 2-dehydrogenase (NADP+) enzyme there by interrupting the biological cycle of *Streptococcus pneumoniae*. Higher positive values for Demethoxycurcumin indicated that it was the most active component against *Streptococcus pneumoniae*. Thus, the key phytochemicals preventing sinusitis caused by *Streptococcus pneumoniae sp.* are Demethoxycurcumin and flavinoids.

CONCLUSION

It was previously known that *Curcuma longa* plant has medicinal action against Sinusitis. Sinusitis is caused by *Streptococcus 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 (Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin), which could have a significant interaction with the enzyme. It was found that Demethoxycurcuminand flavonoids can form strong bond with the enzyme and can successfully inhibit the metabolic cycle of the microbe. Thus, this study could explain that the presence of Demethoxycurcumin and flavinoids provided medicinal values to *Curcuma longa* against sinusitis caused by *Streptococcus pneumoniae*.

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Table 1. Results of CDocking of phytochemicals with ribitol-5-phosphate 2-dehydrogenase (NADP+)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Demethoxycurcumin	10.31	12.29	1.98
2	bisdemethoxycurcumin	37.27	44.07	6.8
3	Flavonoids	24.71	30.37	5.66
4	Tannis	27.02	23.16	3.86
5	Phlobatannis	10.21	11.87	1.66
6	Terpinoids	-33.9	22.47	56.37
7	coumarin	19.06	28.68	9.62

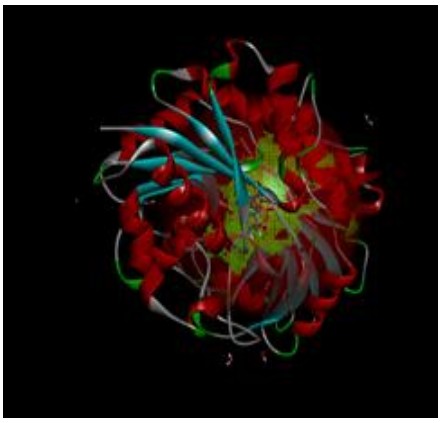

Figure 1. Active site of ribitol-5-phosphate 2-dehydrogenase (NADP+) enzyme

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RESEARCH ARTICLE

Evaluating the Effectiveness of Phytochemicals from *Curcuma longa* (Turmeric) against Diarrhoea through *In-silico* Approach

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ABSTRACT

It has been well established that the prevention of several chronic diseases is possible by the application of natural components. The active composition of Phytochemicals, that are obtained from plants are a key source for such natural constituents. Reports have suggested that *Curcuma longa* plant extract can be usedin curing Diarrhoea. The plant extract from *Curcuma longa* contains several phytochemicals. Phytochemicals are known to play specific pharmacological effects in human health as they have many beneficial effects, such as, anti-inflammatory, anti-allergic, antioxidants, antibacterial, antifungal, chemopreventive, hepato-protective, hypolipidemic, neuroprotective, hypotensive, anti-aging, anti-diabetic. They can also act against osteoporosis, DNA damage, cancer and heart diseases. 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 of *Curcuma longa* 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 Demethoxycurcumin and flavinoids can effectively deactivate the Shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Escherichia coli*.

Key words: phytochemical, BIOVIA, Discovery studio, *Curcuma longa, Escherichia coli*

INTRODUCTION

From the beginning of the human existence, it is known to the mankind that the nature is the key reservoir of resources. We are familiarized ourselves with plants, nature, natural products and have used them in a variety of ways through times. Primitive man in search of food and livelihood began to exploit the use of suitable plants with pharmacological activities, for therapeutic purpose. With time the relationship between plants and human has grown and many plants have been explored or utilized as medicines. The exponential growth in knowledge to treat diseases continued at an accelerating pace and a number of new plant-derived drugs increased vividly. Nature has bestowed our country with an enormous wealth of medicinal plants. Hence, India has often been referred to as the Medicinal Garden of the world. The mention of use of therapeutic plants areseen in Indian Vedas, which were useful in curing different kind of diseases. Now a days, the traditional conventional system of medicine is widely accepted and practiced mostly for treatment by people worldwide. Herbal medicine or phytomedicine is the use of plants and plant products for medicinal and therapeutic purpose for treatment of diseases and to improve human health. The phytochemicals in plants protect them against *microbial* infections. Phytochemicals are active ingredients component which possess therapeutic properties characteristic that are considered as a medicine or drug in clinical aspect [1].

A little caution, small changes in lifestyle and care if taken, can prevent these lifestyle diseases from increasing vigorously. Throughout time, the use of plants and plant-derived products have continued to offer a plethora of health-related benefits. In addition to the rich culinary uses, particularly in spices, some herbs contribute striking towards health benefits also [2]. Some of them are known to exhibit anti-cancerous and antioxidant activities from ancient times [3].The customary use of herbal plants for the treatment of many diseases is associated withthe folk medicine system, from different parts of the world. Natural products from some plants, fungi, bacteria and other organisms, continue to be prevalent as pharmaceutical preparations either as pure compounds or as extracts for treatment purpose. There is a great variety of constituents that can be extracted and characterized from plants [4]. Recent epidemiological evidence reported that the putative beneficial role of intake of fruits and vegetables and other plant products on the risk of diseases of aging may not be exclusively due to these antioxidants, but also from phytochemicals contained in fruits and vegetables [5].

Curcuma longa belonging to the Zingiberaceae family, is a perennial herb that attains a hightup to 1 m high with a short length stem, distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China. In India it is popularly known as “Haldi”, in Malaysia, Indonesia and India it has been well studied due to its economic importance. Its rhizomes are oblong, ovate, pyriform, often short-branched and are a household remedy in several countries [6]. As a powder, called turmeric, it is used for flavouring food products and it also hasdigestive properties along with many beneficial importance [7]. Turmeric contains a list of components like 69.4% carbohydrates, 6.3% protein, 5.1% fat, 3.5% minerals, and 13.1%. moisture. The essential oil (5.8%) obtained by steam distillation consists of Sesquiterpenes (53%), zingiberene (25%), a-phellandrene (1%), sabinene (0.6%), cineol (1%), and borneol (0.5%). Curcumin (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated from turmeric. Curcumin has a melting point of 176–177 °C; and forms a reddish-brown salt with alkali and is soluble in acetic acid, ethanol, alkali, ketone and chloroform [8].

Globally, Acute Diarrheal Disease (ADD), has become a major health concern, particularly in the younger children in resource-limited countries [9]. The etiological agents most frequently involved vary according to the socio economic status and health conditions of the region. Diarrheagenic *Escherichia coli* strains have been reported as the most prevalent etiologic agents for the disease. *E. coli* is included in the group of bacteria of critical priority for the development of new antibiotics. In the search for new alternative therapies for treating ADD, natural products are being evaluated. Among those, curcumin (CUR), the main biologically active curcuminoid of *Curcuma longa*, possesses a wide spectrum of biological activities, such as antioxidant, anti-inflammatory, anti-tumor, anti-

proliferative and anti-protozoal properties. CUR alone or combined with some nanomaterials has demonstrated an antibacterial property against both Gram-positive and Gram-negative bacteria, such as *E. coli* [10]. The diarrheagenic strain of *Escherichia coli* and *Staphylococcus aureus* are known to cause gastrointestinal illness in humans and other animals [11]. The present study was conducted for the identification of the phytochemicals of turmeric responsible for the treatment of diarrhoea.

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 asbioactive components to protect them from pathogens. These plants or their parts are consumed by humans, their phytochemicals fight off threats to health. Majority of phytochemicals have been used in traditional medicines. Published works have reported that *Curcuma longa* contains Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin etc. It has already been established that *Curcuma longa* plant belonging to family Zingiberaceae 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 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*. It has been found that Shikimate dehydrogenase enzyme (protein database code 1NYT) is involved in some metabolism 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 to perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Curcuma longa* plant were downloaded from the website. 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 carriedout with the help of CDocker protocol of BIOVIA software under “receptor-ligand interaction”. The enzyme was considered to be the receptor molecule and the phytochemical as the ligand molecule. 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

Figure. 1 shows the active site of the Shikimate dehydrogenase enzyme. It appears as light green in color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm, which is a grid-based molecular docking method and is 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 best interaction during the docking was chosen based on their high positive value of -CDOCKER energy and on the small difference between -CDOCKER energy and -CDOCKER interaction energy [12]. Table 1 shows that the interaction between Shikimate dehydrogenase and Tannis has the highest positive value of -CDOCKER energy (32.58) and minimum value of the difference (0.71) between - C DOCKER interaction energy and - C DOCKER energy followed by that of phlobatannis, demethoxycurcumin and bisdemethoxycurumin. Thus the results indicated that tannis, phlobatannis, demethoxycurcumin and bisdemethoxycurcumin can effectively deactivate the Shikimate dehydrogenase enzyme there by interrupting the biological cycle of *Escherichia coli*. Higher positive values for tannis indicated that it was the most active ingredient against *Escherichia coli*. On the other hand, phlobatannis, demethoxycurcumin and bisdemethoxycurcumin can deactivate the enzyme to a small extent (negative –Cdocker energy but positive -CDocker interaction energy). Terpinoid cannot interact with Shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing diarrhea caused by *Escherichia coli* are Tannis and phlobatannis.

CONCLUSION

It was previously known that *Curcuma longa* 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 the molecular docking operation was performed for identifying the effective phytochemical (Demethoxycurcumin, flavonoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin), which can have a significant interaction with the vital enzyme (Shikimate dehydrogenase) of the microbe. It was found that Tannis and phlobatann is can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Demethoxycurcumin and bisdemethoxycurcumin were found to be less effective in deactivating the enzyme of the microbe. Terpinoid cannot deactivate the enzyme. Thus, this study could explain that the presence of Tannis and Phlobatannis provided the medicinal values to *Curcuma longa* against diarrhea caused by *Escherichia coli*.

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Table 1. Results of CDocking of phytochemicals with Shikimate dehydrogenase enzyme

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Demethoxycurcumin	10.45	12.52	2.07
2	bisdemethoxycurcumin	27.42	35.11	7.69
3	Flavonoids	17.82	27.43	9.61
4	Tannis	32.58	33.29	0.71
5	Phlobatannis	15.37	17.21	1.84
6	Terpinoids	-40.23	19.98	60.21
7	coumarin	4.76	19.36	14.6

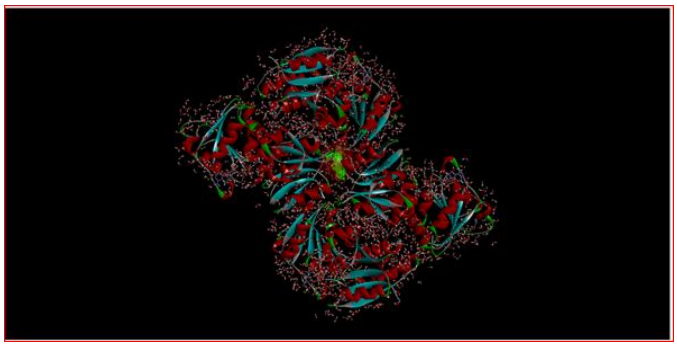

Figure 1. Active site of Shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals Screened from *Ocimum tenuiflorum* against Enoyl-Acyl Carrier Protein Reductase of *Streptococcus pneumoniae* Causing Sinusitis

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ABSTRACT

Sinusitis is among the most common medical conditions that can significantly decrease quality of life, aggravate concomitant conditions and might require consequential medical expenditure. Sinusitis is a bacterial inflammation of paranasal sinuses of the nasal cavitycausing mucus to be filled from within. While the medications that are developed could help treat the case, it is not completely accessible to everyone due to their expensive nature or side effects on individuals. It is presumed that *Ocimum tenuiflorum* plant extract can be used to cure Sinusitis. The molecular docking of the phytochemicals with the microbial 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 Rosmarinic acid can effectively deactivate the enzyme Enoyl-Acyl Carrier Protein Reductase thereby interrupting the life cycle of the microbe.

Key words: phytochemical, BIOVIA, Discovery studio, *Ocimum tenuiflorum*, *Streptococcus pneumoniae*

INTRODUCTION

According to medical strategies reported and published by W.H.O., approximately 30% of the global populations do not have regular access to medicines. In the poorest parts of Africa and Asia, the percent of such crowd goes up to 50% [1]. The population below the poverty line lacks access to such facilities due to higher retail price of artificially synthesized drugs [2]. Moreover, not every individual is same with respect to the metabolic activity taking place within the body. When treated with synthetically designed drugs not everyone gives the same response, in fact some individuals develop a strong reaction toward the components of the drugs. The side effects of drugs may vary from being mild to fatal [3]. Nature has been the genesis for several medicinal agents and modern drugs since the beginning [4, 5]. 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. Phytochemicals are non-nutritive compounds obtained from plants. These organic chemical compounds are the essence of the biotic medicines. Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds [6]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes, etc. [7]. 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 and cost effective.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins. Due to the immense potential of medicinal plants, research needs in the field of medicinal plants are huge. Research into the quality, safety, biological activity and clinical efficacy of these 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 investigating the constituents and determining the biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating their biological activity in a relevant *in vitro* system using animal models [8]. Plants that demonstrate anticancer, antioxidant, anti-inflammatory, immunostimulatory and antimicrobial properties have received research attention.

Tulsi belongs to family Lamiaceae. This plant commonly known as holy basil or tulsi, is an aromatic perennial. It is native to the Indian subcontinent and is widespread as a cultivated plant through out the Southeast Asian tropics. Tulsi is cultivated for religious and traditional medicinal purposes, and for its essential oil. It is widely usage such as in herbal tea, in Ayurveda and is also an important plant in Vaishnava tradition of Hinduism (where worship involving holy basil plants or leaves are performed) [9, 10, 11]. This plant extract contains different phytochemicals that can inhibit the effect of sinusitis caused by *Streptococcus pneumoniae*. Tulsi is known to contain phytochemicals like Rosmarinic acid, Luteolin, Apigenin, Eugenol, Carnosic acid etc.[12, 13]. 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.

A group of bacteria belonging to genus *Streptococcus* generally cause Sinusitis. They are gram-positive, alpha-hemolytic (under aerobic conditions) or beta-hemolytic (under anaerobic conditions), facultative anaerobic members. They are usually found in pairs (diplococci) and do not form spores and are nonmotile [14, 15]. Sinusitis is a common inflammation of the paranasal sinuses, the cavities that produce the mucus necessary for the nasal passages to work effectively. It can be acute or chronic. The paranasal sinuses have the same mucous membrane lining as the nose. They produce a slimy secretion called mucus. This keeps the nasal passages moist and traps dirt particles and germs. Sinusitis occurs when mucus builds up and the sinuses becomes inflamed [16, 17]. This study focuses on the identification of the phytochemical of *Ocimum tenuiflorum*res ponsible to cure Sinusitis caused by *Streptococcus pneumoniae.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used for analysis of the current study. The software makes the use of machine learning techniques to predict the level of molecular interactions and provide the data for the analysis.

List of phytochemicals

Phytochemicals are chemical compounds produced by plants, in order to help them survive from attack by predators or pathogens or competitors. The potential threats to plants may include bacteria, viruses, fungi. When these plants consisting of phytochemicals are consumed by humans the phytochemicals help fight off threats to health. Several works have shown the effectiveness of *Ocimum tenuiflorum* which contains, Rosmarinic acid, Luteolin, Apigenin, Eugenol, Carnosic acid,etc. It has already been established that *Ocimum tenuiflorum* plant belonging to Lamiaceae family has potential to help controlling Sinusitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Sinusitis.

Enzyme found in *Streptococcus pneumoniae*

It has been reported that Sinusitis can be caused as a result of *Streptococcus pneumoniae* infestation. The proliferation of this bacteria requires to proceed with several metabolic pathways. These metabolic cycles need to be regulated by different enzymes for the bacterial survival. BRENDA enzyme database was used to identify and list different enzymes found in *Streptococcus pneumoniae* bacteria. It has been found that Enoyl-Acyl Carrier Protein Reductase enzyme (protein database code 2Z6J) is involved in Fatty acid metabolism (KEGG) and very crucial for survival of *Streptococcus pneumoniae*.

Molecular docking

Molecular docking has become a powerful tool in the drug discovery process. Molecular docking method is used to identify the phytochemicals from plant extracts that act as ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio modules of BIOVIA software was used in the present study for identifying molecular interaction and performs molecular docking. In this process first the SDF files for the phytochemicals present in *Ocimum tenuiflorum* plant were downloaded from websites like PubChem and Mol-Instincts. The protein database code of the Enoyl-Acyl Carrier Protein Reductase enzyme was identified from the RCSB-PDB. The active site of the enzyme was located via “receptor cavity” protocol found under "receptor-ligand interaction" menu. The process for Molecular docking was carried out using the CDocker protocol of BIOVIA software under “receptor-ligand interaction”. The enzyme molecule was considered as the receptor molecule and the phytochemical was considered as the ligand. The “-CDOCKER_ENERGY” and“-CDOCKER_INTERACTION_ENERGY” were used as indicator for analyzing 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 Enoyl-Acyl Carrier Protein Reductase enzyme. The enzyme appears as light green in color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method which is optimized for accuracy. The ligand conformations can be obtained by the Molecular Dynamic methods. -CDOCKER energy can be calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy during nonbonded interaction between the protein and the ligand molecules. The criteria for choicest interaction was chosen based on their a) high positive value of -CDOCKER energy and b)small difference between -CDOCKER energy and -CDOCKER interaction

energy[18]. Table 1 denotes the interaction between Enoyl-Acyl Carrier Protein Reductase and Rosmerinic acid which is at the highest positive value of -CDOCKER energy (50.2859) and minimum value of the difference is (1.522) between - C DOCKER interaction energy and - C DOCKER energy. Based on the results we can interpret Rosmerinic acidto be effective in deactivating the Enoyl-Acyl Carrier Protein Reductase enzyme and hence the biological cycle of *Streptococcus pneumoniae*. Higher positive values for Rosmerinic acid indicated it to be the keycomponent acting against *Streptococcus pneumoniae*. On the other hand, Luteolin, Apigenin, Eugenol, Carnosic acid can deactivate the enzyme to some extent. As both –C DOCKER and –C DOCKER interaction are positive they have significantly less differences between them. Thus, the significant phytochemical that can prevent Sinusitis caused by *Streptococcus pneumoniae* is Rosmarinic acid.

CONCLUSIONS

It was previously known that *Ocimum tenuiflorum* plant has medicinal action against Sinusitis. Sinusitis 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 Rosmarinic acid, Luteolin, Apigenin, Eugenol, Carnosic acidetc, which can have a significant interaction with the vital enzyme Enoyl-Acyl Carrier Protein Reductase of the *Streptococcus pneumoniae*. It was found that (Rosmerinic acid) can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the *Streptococcus pneumoniae*. Thus, this study could explain that the presence of Rosmarinic acid, Luteolin, Apigenin, Eugenol, Carnosic acid provided the medicinal values to *Ocimum tenuiflorum* against Sinusitis caused by *Streptococcus pneumoniae*. Out of which Rosmarinic acid analysed to be more effective.

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Table1. Results of CDocking of phytochemicals with Enoyl-Acyl Carrier Protein Reductase enzyme

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Rosmarinic acid	50.2859	51.8079	1.522
2	Luteolin	31.2444	37.2832	6.0388
3	Apigenin	29.1283	37.0727	7.9444
4	Eugenol	12.9607	25.1892	12.2285
5	Carnosic acid	9.55462	27.2762	17.72158

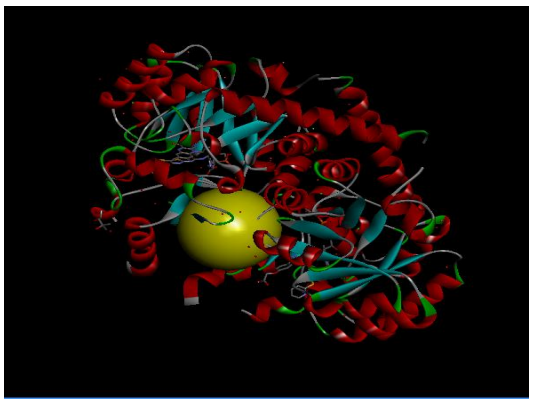

Figure 1. Active site of (Enoyl-Acyl Carrier Protein Reductase) enzyme

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RESEARCH ARTICLE

Analysis of Binding and Interaction of Phytochemicals from *Curcuma longa* (Turmeric) against Ulcer: An *In silico* Virtual Screening Approach

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ABSTRACT

Phytochemicals are bioactive, non-nutritive compounds components from plants sources, that sustain or promote health. Several reportshave suggested that *Curcuma longa* plant extract could have benefits in treatment of ulcer. The plant extract from *Curcuma longa* contains a number of phytochemicals in it. Ulcer is caused by *Helicobacter pylori* in which one of the key enzymes involved in its biochemical pathway is Cinnamyl alcohol dehydrogenase (NADP+). In the current study, the molecular docking of the enzyme from *Helicobacter pylori* was carried out with the phytochemicals from *Curcuma longa* using BIOVIA Discovery Studio. The strength of the interaction was calculated based on -CDocker energy and -CDocker interaction energy, along with their difference. Values skewed towards the positive digits, for both the parameters indicated that out of different phytochemicals Demethoxycurcumin and flavinoids can effectively deactivate the Cinnamyl alcohol dehydrogenase enzyme there by interrupting the life cycle of *Helicobacter pylori*.

Key words: phytochemical, BIOVIA, Discovery studio, *Curcuma longa*, *Helicobacter pylori*

INTRODUCTION

The current life style changes of people are seen to be responsible for prevalence of several diseases these days. Changing work conditions, less physical activities, jobs leading to sedentary habits along with comfortable life and unhealthy eating habits has exposed us to many diseases and have increased the stress associated with our lives. With the steep increase in prevailing diseases, the need for treatment of such diseases has also increased. As, most of the synthetic drugs are known to have several side effects along with treating the disease condition efficiently, hence the mankind these days have focused on to natural remedies for treatment of such diseases. Phytomedicine is the best use of plants for its medicinal value and therapeutic intent in curing of several diseases and of the betterment of human health. Secondary metabolites of plants are called as phytochemicals. These compounds act as insecticides. Phytochemicals are active ingredients which have a lot of therapeutic properties that are considered as a herbal medicine or as a drug [1].

Pharmacological studies have shown that intake of fruits and vegetables as well as other plant products are helpful to diminish the risks associated with diseases. According to World Health Organization, medicinal plants would be the meritorious source in obtaining different types of drugs. More than 80% of individuals from developed countries depend on conventional medicines, whose components are derived from plants sources. This suggests that plants would be a source of immense potentials and could be investigated thoroughly for understand their therapeutic properties, safety and efficiency. Various medicinal plants have been used for years on a regularbasis to improve the life quality of people all around the world [2].

Pathogenic microorganisms can cause number of diseases by their harmful quality of invasiveness and toxic character. Synthetic antibiotics used against them can cause oxidative stress along with damage to the DNA, proteins and lipids in human cells, the consequences of which could be life threatening at times. Due to the presence of phytochemicals and antioxidants, plants can pave a way for development of antimicrobial drug [3]. Phytochemicals contribute to human health as in many ways asthey have antioxidants, antibacterial, antifungal, anti-inflammatory, anti-allergic, antispasmodic, chemo preventive, hepatoprotective, hypolipidemic, neuroprotective, hypotensive properties. They can also help prevent aging, diabetes, osteoporosis, cancer and cardiac diseases. They can also be effective in inducing apoptosis, diuretic, CNS stimulant, analgesic and can protect humans from UVB-induced carcinogenesis, immuno-modulator and carminative effects [4]. The medicinal values of these plants lie in some chemical components that produces a strong physiological action on the human health. The most important of these bioactive substances present in plants are alkaloids, tannins, flavonoids, and phenolic compounds [5].

Many of the indigenous medicinal plants areused as spices in cooking for their beneficial role. They are also sometimes added to food for pregnant women and nursing mothers for medicinal purposes [6]. For centuries together, people have turned to natural remedies for treatment of common ailments such as colds, allergy, stomach upset, toothaches and this tradition is constantly increasing. Thus, there has been a shift in universal trend tradition from synthetic to herbal and traditional medicines, which we can say 'Return to Nature' for the prevention as well as cure of diseases and ailments [1]. India has a rich history of use of plants for therapeutic purposes. Turmeric (*Curcuma longa*) is one among such medicinal plant which extensively isused in Ayurveda, Unani and Siddha medicine as home remedy for several diseases. *C. longa L.*, botanically is related to ginger and belongs to the Zingiberaceae family. It is a perennial plant having a short stem with large oblong leaves and bears ovate, pyri form or oblong rhizomes, which are often branched and brownish-yellow incolour.

Turmeric rhizome is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia. It is also considered to be auspicious and is a part of religious customs. In old Hindu medicine, it has found extensive use for the treatment of sprains and swellings caused by injury. Even these days, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough,

diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have been isolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant, anti-inflammatory, wound healing, anticancer and antibacterial activity [2]. It has also found use in conditions such as inflammation, ulcer and cancer. It also has antifungal, antimicrobial renal and hepatoprotective activities [7]. Turmeric contains a number of chemical substances such as protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). Phenolic diketone, curcumin (diferuloylmethane) (3-4%) is responsible for the brownish yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%). Other phenolic diketones demethoxycurcumin and bis-demethoxycurcumin have also been isolated from the rhizomes of *C. longa* [8].

A group of bacteria belonging to genus *Helicobacter* generally cause ulcer. They are helical shaped Gram-negative bacteria and are highly pathogenic. *H pylori* is considered to be the primary cause of non-NSAID induced peptic ulcer disease, with an estimated prevalence of 95% in patients with duodenal ulcers and 84% inpatients with gastric ulcers [9]. This study focuses on the identification of the Phytochemicals from *Curcuma longa* that are responsible 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 conducting the study. The prediction of the level of molecular interaction, machine learning technique is utilized by the software.

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 and other pathogenic microorganisms. When these plants or their parts are consumed by humans, they help fight off threats to human health and hence are useful as traditional medicine. Published works have reported that *Curcuma longa* contains a number of phytochemicals as their bioactive components such as, Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin. It has already been established that *Curcuma longa* plant belonging to family Zingiberaceae has potential to help curing ulcer. This work is focused on identification of the particular Phytochemicals that are capable of inhibiting and curingulcer caused by *Helicobacter pylori*.

Enzyme found in *Helicobacter pylori*

It has been reported that ulcer can be caused as a result of *Helicobacter* infestation. Various metabolic cycles are known to be involved in the life cycle bacteria for their survival. These metabolic cycles are regulated by different types of enzymes. BRENDA and RCSB database were used to find outand list different enzymes found in *Helicobacter*. It has been found that Cinnamyl alcohol dehydrogenase enzyme (protein database code 3 TWO) is involved in some metabolism and is very crucial for the survival of the particular micro-organisms.

Molecular docking

Molecular docking technique has been used to identify the phytochemical from the plant extract, that could act as ligand to form a strong covalent bond with the bacterial protein for successfully inhibitingit. The Discovery studio module of BIOVIA software was used for identifying molecular interactions and to perform molecular docking. In this process first the sdf files for the phytochemicals found in *Curcuma longa* were downloaded. The protein database code of Cinnamyl alcohol dehydrogenase enzyme was identified. Further, the active site of the enzyme was identified via “receptor cavity” protocol found under "receptor-ligand interaction" menu. Molecular docking was carried out using 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 major phytochemical responsible for curing the disease.

RESULTS AND DISCUSSION

Figure 1 shows the active site of Cinnamyl alcohol dehydrogenase enzyme. It appears light green in color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method that is optimized in terms of 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. For the selection of best interaction values highly skewed towards the positive side of -CDOCKER energy and a minimum difference between -CDOCKER energy and -CDOCKER interaction energy were preferred [10]. Table 1 shows that the interaction between Cinnamyl alcohol dehydrogenase and Demethoxycurcumin showed the highest positive value of -CDOCKER energy (13.55) and minimum value of the difference (2.03) between - C DOCKER interaction energy and - C DOCKER energy followed byphlobatannis and tannis. Thus, the results indicate that Demethoxycurcumin, phlobatannins and tannis can effectively deactivate the Cinnamyl alcohol dehydrogenase enzyme there by interrupting the biological cycle of *Helicobacter pylori*. Higher positive values for Demethoxycurcumin indicated that it was the most active ingredient against *Helicobacter pylori*. Thus, the key phytochemicals preventing ulcer caused by *Helicobacter pylori* are Demethoxycurcumin, phlobatannis and tannis.

CONCLUSIONS

It was previously reported that *Curcuma longa* plant has therapeutic 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 (Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin), which can have a significant interaction with the vital enzyme Cinnamyl alcohol dehydrogenase of the microbe. It was found that Demethoxycurcumin, phlobatannis and tannis can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.Thus, this study could explain that the presence of Demethoxycurcumin, phlobatannis and tannis provided the medicinal values to *Curcuma longa* against diarrhea caused by *Helicobacter pylori*.

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Table 1. Results of CDocking of phytochemicals with Cinnamyl alcohol dehydrogenase

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	Difference between -CDOCKER interaction energy and - C DOCKER energy
1	Demethoxycurcumin	13.55	15.58	2.03
2	bisdemethoxycurcumin	36.48	45.79	9.31
3	Flavonoids	25.56	33.95	8.39
4	Tannis	32.91	30.01	2.9
5	Phlobatannis	13.11	14.79	1.68
6	Terpinoids	-24.52	31.62	56.14
7	coumarin	20.12	29.72	9.6

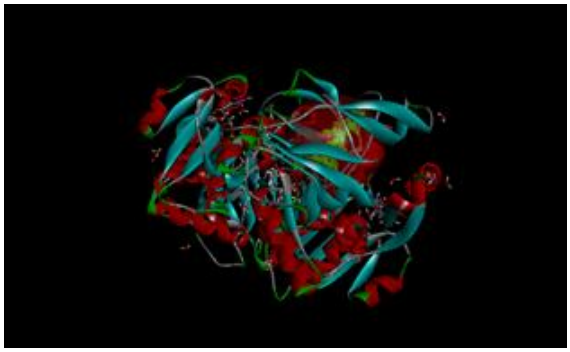

Figure 1. Active site of Cinnamyl alcohol dehydrogenase enzyme

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RESEARCH ARTICLE

In-silico Molecular Docking Studies and Anti-Microbial Activity of Phytochemicals from *Jasminum sambac* against Gingivitis

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ABSTRACT

Plants are an essential source of nutrients and health-beneficial components which are crucial for human health.It has been reported that *Jasminum sambac* plant extract could be usefulin the cure of Gingivitis. This plant extract contains different phytochemicals such as, ducosterol, hesparidin, dotriacotanoic acid, etc. Gingivitis is caused by *Treponema denticola.* One of the key enzymes which is involved in its biochemical pathway is phosphoribosylaminoimidazole carboxylase. The molecular docking of this phytochemical with the enzyme from *Treponema denticola* was studied using BIOVIA Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. The values skewed towards positive side for both the parameters indicated that out of different phytochemicals dotriacotanoic acid and dotriacotanol can effectively deactivate the phosphoribosylaminoimidazole carboxylase enzyme there by interrupting the life cycle of *Treponema*.

Key words: phytochemical, BIOVIA, Discovery studio, *Jasminum sambac, Treponema denticola*.

INTRODUCTION

In the modern world, health issues have become a major concern due to the life style adopted by majority of people. The present approach of people towards their health has led to the exponential growth in diseases. It has been

demonstrated that vegetables, fruits and grains exert a nutritive as well as protective effect against several diseases. This protective role could be attributed to the phytochemicals in them, which have defined, bioactive, non-nutrient components. Several phytochemicals have been identified till date, and still a large percentage of them remains to be unknown. These identified phytochemicals include tannins, flavones, triterpenoids, steroids, saponins, andalkaloids [1]. Regular diet provides us the nutritional supplies for our life and growth, even some components in the diet can exert valuable effects when consumed regularly. These components with beneficial effects are called as "functional foods" or "nutraceuticals" [2]. These components, with a wide range of composition and functionality, are capable of providing different beneficial effects beyond just nutrition and hence result in improved health conditions. Phytochemicals present in plants are bioactive, non-nutrient compounds present in vegetables, fruits, grains whose ingestion helps in reduction in the risk of major diseases [3].

Nature has been a source of medicinal agents from ages, an impressive number of synthetic drugs have been derived from these sources [4]. Medicinal plants are seen to havea distinct physiological action on the human body which is beneficial in terms of health. The substance that causesuch beneficial effects are the phytochemicals that are present in plants.Varieties of medicinal plants are spread all over the globe and majority of them are used for the preparation of drugs. In fact, different parts of the plants can be used for therapeutic purpose, such as their leaves, bark, flower, fruits, root, tuber, rhizome, bulb, etc [5]. A vast majority of population particularly those living in villages depend largely on such herbal medicines. Scientific documentation of several such medicinal plants has been well established and investigated [6]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, etc. [7]. Medicinal plants play a key role in human health care.Ayurveda is a traditional Indian medicinal system being practiced for ages beyond. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants [8].

From ancient time many plants are used as food and to flavor foods and also to treat health disorders by preparing medicines [9]. Many of the medicinal plants are used as spices and food items. The effective extraction procedure and proper assessment of their antioxidants have applications as therapeutics [10]. Medicinal plants are the foundation of several drugs prescribed by modern physicist. More than 25% of modern pharmaceutical drugs have botanical origins. Research needs in the field of medicinal plants are huge. In fact, research into the quality, safety, biological activity and clinical efficacy of the numerous plants in common usage is of need now. Newly emerging scientific techniques and approaches are used for investigating the constituents and to determine the 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.

Jasmine belongs to family Oleacae. Jasmine flower extract could be used in the cure of Gingivitis [11]. Jasmine is known to contain phytochemicals like 2-phenylethyl beta-primeveroside, benzyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside, ducosterol, dotriacontanol, hesparidin, dotriacontanoic acid, iridoidal glycosides, oleanolic acid, etc. There is high possibility that these phytochemicals play a major role in curing Gingivitis. However, there is no report identifying the specific phytochemical responsible to cure Gingivitis. A group of bacteria belonging to genus *Treponema* generally cause Gingivitis [13]. They are rod-shaped Gram-negative bacteria. *Treponema denticola* infection is a common bacterial disease that affects orally. Humans become infected most frequently through contaminated water or food. This study focuses on the identification of the phytochemical from *Jasminum sambac* responsible for the cure of Gingivitis caused by *Treponema denticola.*

MATERIALS AND METHODS

Software used

Discovery studio module of BIOVIA software (Dassault Systemes of France) was used to carry out the present study. Machine learning technique is utilized by the software in order to predict the level of molecular interactions between the ligand and the receptor.

List of phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. When these plants or their parts are consumed by humans, the phytochemicals present in them help fight threats to human health and hence they are useful as traditional medicine. Published works have shown that *Jasminum* contains 2 phenylethyl beta-primeveroside, benzyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside, ducosterol, dotriacontanol, hesparidin, dotriacontanoic acid, iridoidal glycosides, oleanolic acid, etc. It has already been established that *Jasminum* plant belonging to Oleacae family has potential to help controlling Gingivitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Gingivitis.

Enzyme found in *Treponema denticola*

It has been reported that Gingivitis can be caused as a result of *Treponema denticola.* infestation. Various metabolic cycles in the bacteria are responsible for the survival of it. These metabolic cycles are regulated by different enzymes. BRENDA enzyme database was used to identify and list out the various enzymes found in *Treponema denticola*. It has been found that phosphoribosylaminoimidazole carboxylase enzyme (protein database code 5C5D) 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 acts as a ligand and forms 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 to perform molecular docking. During the process, first the sdf files for the phytochemicals found in the *Jasminum sambac* were downloaded. The protein database code of phosphoribosylaminoimidazole carboxylase enzyme was identified. 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

Figure 1 shows the active site of phosphoribosylaminoimidazole carboxylase 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, that is 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 [13]. Table 1 shows that the interaction between phosphoribosylaminoimidazole carboxylase and dotriacontanoic acid interaction has the highest positive value of -CDOCKER energy (57.20) and minimum value of the difference (0.59) between -CDOCKER interaction energy and -CDOCKER energy; along with interaction between phosphoribosylaminoimidazole carboxylase dotriacontanol interaction has the highest positive value of -CDOCKER energy (52.46) and minimum value of the difference (0.46) between -CDOCKER interaction energy and -CDOCKER energy. Thus, the results indicated that dotriacotanol can effectively deactivate the phosphoribosylaminoimidazole carboxylase enzyme thereby interrupting the biological cycle of *Treponema denticola*. Higher positive values for dotriacotanol indicated that it was the most active ingredient against *Treponema denticola*. On the other hand, hesparidin, iridoidal glycoside, oleanolic acid can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). 2 phenylethyl beta permeveroaide, benzyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside and ducosterol cannot interact with phosphoribosylaminoimidazole carboxylase enzyme. Thus, the key phytochemicals preventing Gingivitis caused by *Treponema denticola* isdotriacotanol.

CONCLUSION

It is already stated that *Jasminum* plant has medicinal action against Gingivitis. Gingivitis is caused by *Treponema denticola.* 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 phenylethyl beta-primeveroside, benzyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside, ducosterol, dotriacontanol, hesparidin, dotriacontanoic acid, iridoidal glycosides, oleanolic acid) which can have a significant interaction with the vital enzyme (*phosphoribosylaminoimidazole carboxylase*) of the microbe. It was found that dotriacontanoic acid and dotriacontanol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Hesparidin, iridoidal glycosides, oleanolic acid were found to be not much effective in deactivating the enzyme of the microbe. 2 phenylethyl beta-primeveroside and benzyl-6-o-beta D-xylopyranoside cannot deactivate the enzyme. Thus, this study could explain that the presence of dotriacontanoic acid and dotriacontanol provided the medicinal values in *Jasminum* against Gingivitis caused by *Treponema denticola.*

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Table 1. Results of CDocking of phytochemicals with phosphoribosylaminoimidazole carboxylase enzyme

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Dotriacotanoic acid	57.20	56.61	0.59
2	Dotriacotanol	52.46	52.92	0.46
3	Hesparidin	-2.52	51.47	53.99
4	Iridoidal glycosides	-113.90	71.19	185.09
5	Oleanolic acid	-66.45	25.64	92.09
6	Ducosterol	Failed	Failed	NA
7	Benzyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside	failed	Failed	NA
8	2 phenylethyl beta-primeveroside	failed	Failed	NA

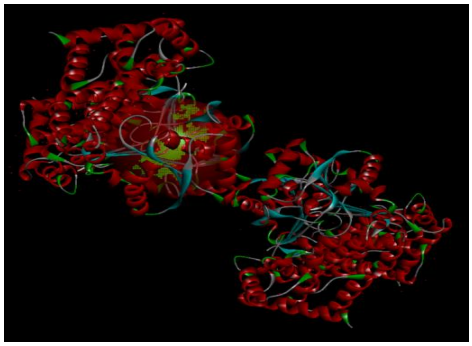

Figure 1. Active site of phosphoribosylaminoimidazole carboxylase enzyme

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RESEARCH ARTICLE

An *In silico* Analysis of Phytochemicals as Antimicrobial Agents from *Pogostemon cablin* against Eczema

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ABSTRACT

Phytochemicals are non-nutritive compounds that are obtained from plants sources. Due to the production of diversity of phytochemicals with beneficial properties, the human civilization has been dependent on them for centuries. These synthetic antimicrobial compounds have founda wide use in prevention and cure of microbial diseases. It has been reported that *Pogostemon cablin* extract could have use in the cure of Eczema. This plant extract contains different phytochemicals. Eczema, that is caused by *Staphylococcus aureus* is otherwise called as dermatitis. One of the key enzymes involved in its biochemical pathway is the penicillin-binding protein 4 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. Higher values skewed to the positive side indicated that out of various phytochemicals studied, tilianin and retusin can effectively deactivate the penicillin-binding protein 4 enzyme thereby interrupting the life cycle of *Staphylococcus aureus*.

Key words: phytochemical, BIOVIA, Discovery studio, *Staphylococcus aureus*, *Pogostemon cablin*.

INTRODUCTION

Due to theadverse effects of polluted environment, the human health conditions have started declining. Certain people remain unhealthy due to the choice of their detrimental life style, such as, consuming alcohol, drugs, etc. WHO has estimated millions of people to be suffering from neurological disorders, physiological problems related to alcohol as well as drug abuse. The unhealthy surrounding including environmental pollution, smoke of cigarettes, unnecessary use of pesticides and many more affects us both mentally and physically [1]. Due to these unhealthy lifestyles, diseases such as heart diseases, diabetes and many more have become prevalent [2]. India is known for its dependence on traditional medicines for several diseases. TheAtharv Veda describes about several diseases, their treatments and drug preparation using medicinal plants[3].

Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, etc.Many researches on medicinal plants contain several vitamins, many phytochemicals which have been helpful for researchers to prepare drug for numerous diseases. Thus, medicinal plants play a key role in human health care. A research shows that about 80% of world population use many medicinal plants for the preparation of different drugs to treat many diseases. This is due to the fact that medicinal plants plentily available and have less or no side effects [5]. Many of the medicinal plants are used as spices in food items, to keep diseases at bay. Many medicinal plants are also used in the preparation of different medicines such as allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. Among different sources of natural products, plants are considered as novel chemical source, that serve as starting material for a number of pharmaceutical products. Medicinal plants are the foundation of many drugs prescribed today by many Pharmacist. More than 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 by enormous size of the market. Research into the quality, safety, biological activity and clinical efficacy of numerous plants in common usage is required. Newly emerging scientific techniques and approaches have shown potentials in the growing area of medicinal plant research, mainly for the purpose of investigatingthe constituents and for the determination of biological activity of medicinal plants. Evidences for the beneficial effects of selected plants is generally based on experiments demonstrating biological activity in a relevant *in vitro* bioassay or experiment using animal models [6]. Plants that demonstrated antimicrobial, anticancer, antioxidant, anti-inflammatory and immunostimulatory properties have received much attention in terms of research.

Pogostemon cablin belongs to family Lamiaceae, its leaves extractsare used to cure disease like eczema [7]. *Pogostemon cablin* is known to contain phytochemicals likeTilianin, stegmasterol, Retusin, Stigmasterol-4-en-3-one, patchoulene, seychellene,etc. There is high possibility that these phytochemicals play a major role in curing eczema. A group of bacteria belonging to genus *Staphylococcus* generally cause eczema. Eczema infection is a common bacterial disease that affects the skin. *Staphylococcus aureus* typically live in animal and human intestines and are shed through faeces. 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 eczema 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 any of their parts consisting of phytochemicals are consumed, they help fight off threats to human health. Several phytochemicals are hence useful as have been used as traditional medicine. Several reports have suggested that *Pogostemon cablin* contains tilianin,

retusin, stigmasterol, seychellene, patchoulene, stigmasterol-4-en-3-one, etc. It has already been established that *Pogostemon cablin* belonging to Lamiaceae family has potential to help inhibit candidiasis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling eczema.

Enzyme found in *Staphylococcus aureus*

It has been reported that eczema can be caused as a result of *Staphylococcus aureus* infestation. Various metabolic cycles in the bacteria, have essential role in its survival. The metabolic cycles of such bacteria are regulated by several enzymes. BRENDA enzyme database was used to identify and list different enzymes found in *Staphylococcus aureus*. It has been found that penicillin-binding protein 4 enzyme (protein database code 5TW8) is involved in glycerolipid metabolism (KEGG) and is very crucial for the survival of the bacteria.

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 cablin* plant were downloaded. The protein database code of penicillin-binding protein 4 enzyme was identified. 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

Figure 1 shows the active site of the penicillin-binding protein 4 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 thehigher positive value of -CDOCKER energy as well as on the minimum difference between -CDOCKER energy and -CDOCKER interaction energy [8]. Table 1 shows that the interaction between penicillin-binding protein 4 and Tlianin has the highest positive value of -CDOCKER energy (20.47) and minimum value of the difference (21.83) between -CDOCKER interaction energy and -CDOCKER energy followed by Retusin with -CDOCKER energy (21.94) and minimum value of the difference (21.85) between -CDOCKER interaction energy and -CDOCKER energy. Thus, the results indicated that Tilianin andRetusincan effectively deactivate the penicillin-binding protein 4 enzyme thereby interrupting the biological cycle of *Staphylococcus aureus*. Positive values for Tilianin indicated that it was the most active ingredient against *Staphylococcus aureus*. On the other hand, stegmasterol, patchoulene, seychellene, stigmast-4-en-3-one can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing eczema caused by *Staphylococcus aureus* are Tilianin, Retusin.

CONCLUSIONS

It was previously known that *Pogostemon cablin* plant 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 phytochemical (Tilianin,Retusin,stigmasterol-4-en-3-one,patchoulene, seychellene, stegmasterol), which can have a significant interaction with the vital enzyme (penicillin-binding protein 4) of the microbe. It was found that Tilianin and Retusin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Seychellene, stigmasterol, patchoulene were not much effective in deactivating the enzyme of the microbe. Thus, the present study suggests that the presence of Tilianin, Retusin provided the medicinal values to *Pogostemon cablin* against eczema caused by *Staphylococcus aureus.*

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Table 1. Results of CDocking of phytochemicals with penicillin-binding protein 4 enzyme

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Tilianin	20.47	42.30	21.83
2	Retusin	21.94	43.79	21.85
3	Stigmasterol-4-en-3-one	-18.85	49.80	68.65
4	Stigmasterol	-33.52	47.06	80.58
5	Seychellene	-40.62	23.80	64.42
6	Patchoulene	-52.51	25.57	78.08

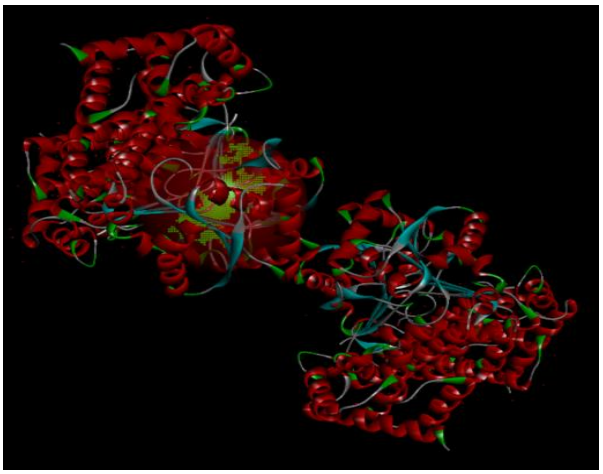

Figure 1. Active site of penicillin-binding protein 4 enzyme

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals from *Chrysopogon zizanioides* (Vetiver) against Malaria Parasite

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ABSTRACT

Chrysopogon zizanioides (L.) Roberty commonly known as vetiver is a perennial bunchy herbaceous species of the Poaceae family. The phyto-extract contains a number of phytochemicals with effective control against malaria caused by *Plasmodium vivax sp.*. One of the key enzymes involved in its biochemical pathway is L-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. Anthraquinone yield a high positive values for both the parameters indicated among the other reported phytochemicals namely, alpha-vetivones, beta-vtivones, cloven and Vetiselinenol. Anthraquinone effectively deactivated the L-lactate dehydrogenase enzyme thereby interrupting the life cycle of *Plasmodium vivax.*

Keywords: Phytochemical, BIOVIA, Discovery studio, *Chrysopogon zizanioides, Plasmodium vivax*.

INTRODUCTION

Medicinal plants loaded with antimicrobial agents and are widely used either directly as folk remedies or indirectly in the preparation of modern pharmaceutical by all sections of the population. World Health Organization (WHO) noted that more than 80% of the world's population depends on traditional and alternative medicine for primary healthcare. However, from prehistoric times various ailments and infectious diseases have been treated by herbal medicines for the betterment of mankind all over the world. Thus, scientists are increasingly showing their interest to develop natural products, either in pure compounds form or as standardized phytot extracts, looking for new leads to develop better drugs against microbial infections (Gaikwad et al. 2012). India has been identified as one of the top

12 mega-diversity centers in the world, with immensely rich medicinal and aromatic plants occurring in diverse ecosystems. These medicinal plants are used both for primary healthcare and for treating chronic diseases such as AIDS, cancer, hepatic and cardiac disorders and age-related diseases such as memory loss, osteoporosis and diabetes. The use of medicinal plants and other natural products with therapeutic properties is as ancient as human civilization and for centuries natural products were the main source of drugs. About 25% of the drugs prescribed worldwide still come from plants, with 121 such compounds still currently in use. At the beginning of the 21st century, 11% of the 252 drugs considered basic and essential by the WHO were exclusively of flowering plant origin. In recent years, there has been serious rethinking on the use of medicinal plants and traditional medicines as a source of new bioactive compounds for the treatment of various ailments (Ahmad et al. 2008).

Chrysopogon zizanioides (L.) Roberty commonly known as vetiver is a perennial bunchy herbaceous species of the Poaceae family that develops in almost every soil type. The stems being stiff, vetiver tufted grass can attain up to 2 m height (Burger et al. 2017). Native to India, vetiver was disseminated around the world some 100 years ago and is since widely cultivated in tropical regions for many different purposes in Haiti, India, Indonesia, and Reunion Island (Burger et al. 2017). *C. zizanioides* have many ethnopharmacological properties like carminative, diuretic, diaphoretic and emmenagogue. The phyto extract had also anti-parasitic and anti-helminhth Properties (Dhawan et al.2016).

Phyto chemicals remain an important source to combat infectious diseases in the world. The alternative and traditional medicines play a vital role to cover the basic health needs in the developing countries. The medicinal properties of these plants is due to the chemicals or secondary metabolites produced by the plants which have definite physiological action on the human body (Shihabudeen et al, 2010). Traditional medicines have been used to treat malaria for thousands of years and are the source of the two main groups, artemisinin and quinine derivatives, of modern antimalarial drugs. With the problems of increasing levels of drug resistance and difficulties in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment (Willcox et al. 2004). Half the world's population is estimated to be at risk for malaria caused by *P. vivax* 1 (Odugbemi et al. 2007). According to the reported studies on *C. zizanioides*, popularly known as khus grass or miracle grass, is an important aromatic plant commonly found in India can be an alternative medicine in treatment malaria infection. This study focuses on the identification of the phytochemicals of *C. zizanioides* responsible to cure Malaria caused by *Plasmodium sp.* by Molecular docking Method.

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

C. zizanioides contains Alpha-vetivones, Anthraquinone, Beta-vetivones, Cloven and vetiselinenol etc. It has already been established that *C. zizanioides* plant has potential to help controlling Malaria. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Malaria. The sdf files for the phytochemicals found in the *C. zizanioides* plant were downloaded from the PUBCHEM database.

Enzyme Used

Brenda enzyme database was used to identify and list different enzymes found in *Plasmodium vivax sp.* bacteria. It has been found that L-lactate dehydrogenase enzyme (PDB ID 5HS4) has been downloaded from the RCSB database, is involved in some metabolism and very crucial for survival of the particular microbe is used in the study.

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. 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 L-lactate dehydrogenase enzyme has been represented in Figure 1. 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 (Brinda et al. 2019). I has been represented that L-lactatedehydrogenase-Anthraquinone interaction has the highest positive value of -CDOCKER energy (15.01) and minimum value of the difference (5.86) between - C DOCKER interaction energy and -C DOCKER energy. Thus the results indicated that anthraquinone can effectively deactivate the L-lactate dehydrogenase enzyme there by interrupting the biological cycle of *Plasmodium vivax sp.*. Higher positive values for anthraquinone indicated that it was the most active ingredient against *Plasmodium vivax sp.*. Alpha-vetivones, beta-vtivones, cloven and Vetiselinenol cannot interact with L-lactate dehydrogenase enzyme. Thus, the key phytochemicals preventing Malaria caused by *Plasmodium vivax sp.* are anthraquinone acid and harmalol.

CONCLUSIONS

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-vetivones, Anthraquinone, Beta-vetivones, Cloven and vetiselinenol), which can have a significant interaction with the vital enzyme (L-lactatedehydrogenase) of the microbe. From this above study it has been concluded that anthraquinone can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha-vetivones, Beta-vetivones, Cloven and vetiselinenol could not deactivate the enzyme. Thus, this study could explain that the presence of anthraquinone acid provided the medicinal values to *C. zizanioides* against Malaria caused by *Plasmodium Sp.*

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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	Alpha-vetivones	-28.14	24.31	52.45
2	Anthraquinone	15.01	20.87	5.86
3	Beta-vetivones	-38.42	24.42	62.84
4	Cloven	-27.1	23.28	50.38
5	Vetiselinenol	-22.82	23.18	46

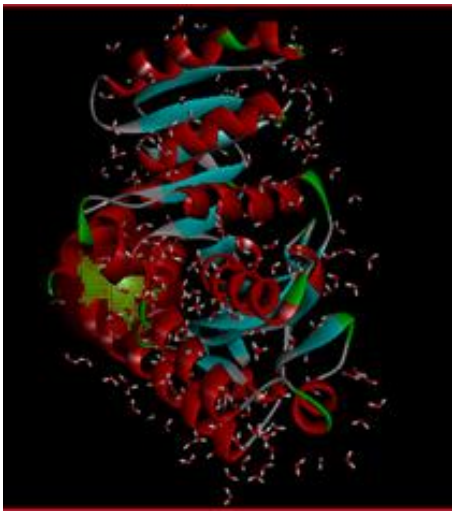

Figure 1. Active site of L-lactatedehydrogenase enzyme

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RESEARCH ARTICLE

Anti-Malaria Activities of Phytochemicals from *Theobroma cacao*: An *In silico* Study

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ABSTRACT

The use of bioactive agents present in phytochemicals to meet health care needs has greatly increased worldwide. Medicinal plants are rich source of phytochemicals that offer traditional, cost effective, less side effect therapy. It has been reported that *Theobroma cacao* plant extract is used for the treatment of malaria. Phytochemicals are the natural plant derived compounds with therapeutic activities such as anticarcinogenic, antimutagenic, antiinflamatory, and antioxidant properties. Malaria is caused by *Plasmodium* parsites. One of the key enzymes involved in its biochemical pathway is L-lactate dehydrogenase. Phytochemicals inhibits the action of enzyme by deactivating the biochemical machinery of the 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 Phloretic acid can effectively deactivate the enzyme L-lactate dehydrogenase thereby interrupting the life cycle of *plasmodium.*

Key words: phytochemicals, Biovia, Discovery studio, *Theobroma cacao*, *Plasmodium.*

INTRODUCTION

Phytochemicals are thenon-nutritive chemical compounds produced by the plants having protective or disease preventive property. They are produced by the plants through primary or secondary metabolism**.** A number of phytochemicals has been extracted from the plant extract**.** Since time immemorial plants have been used as novel

source and reservoir of chemical agents with great restorative activities [1]. Traditionally plants are used as substitute drugs for various ailments affecting humankind. The information on medicinal value of plants conventionally was passed from generation to generation. This passing of information somehow has led to preservation of the knowledge, however the trend is changing with many communities abandoning their cultural practices, this therefore creates the need for the documentation of the information on traditional medicine in both the traditional way and also provide scientific rationalization in order to increase the confidence on the use of plants as alternative means of treatment [2]. 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.

Medicinal plants have been tested extensively and found to have great pharmacological uses such as, anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anti-cancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction and hypolipidemic activity [3]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [4]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. 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. 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. 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.

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. 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 [5]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Cocoa or cacao is an ancient crop having been harvested and used by the indigenous people of Central and South America for thousands of year. Cocoa was introduced to Europe during the 16th century. Cocoa belongs to the family Malvaceae is worldwide known for its beans used in the manufacture of chocolate [6].Cocoa extract is used to cure disease like malaria. Cocoa is known to contain phytochemicals like procyanidin, Quercetin, Syringic acid, Theobromine, Phloretic acid, Naringenin, Luteolin, Orientin, Phenyl acetic acid, Phloretic acid, etc.[7]. There is high possibility that these phytochemicals play a major role in curing malaria. However, there is no report identifying the specific phytochemical responsible to cure malaria.

A group of organisms belonging to genus *Plasmodium* generally causes malaria. *Plasmodium falciparum*, the most virulent of the four human *Plasmodium* species causing malaria, is potentially life-threatening, increasing in prevalence and becoming even more resistant to in-use drugs [8]. They are the unicellular protozoan parasite of humans, and the deadliest species of *plasmodium* that causes malaria in humans. There are six species of *plasmodium* which create significant health threat for humans and they are *P. Vivax, P.falciparum, P. Ovale, P.malariae, P.wallikeri, and P.knowlesi. Plasmodium falciparum* is usually considered the most important in terms of deaths. *P. vivax* is a major

cause of illness across large parts of the world, and it is increasingly argued that deaths, due to this parasite, have been underestimated. *P. ovalecurtisi, P. ovale, p. wallikeri, and P. malariae* are much less common causes of significant disease. The parasite is transmitted through the bite of a female Anopheles mosquito and causes the disease's most dangerous form calleds *Falciparum malaria*. The parasite can be spread to humans through the bites of infected mosquitoes. This study focuses on the identification of the phytochemical of *Theobroma cacao* responsible to cure malaria caused by *Plasmodium falciparum.*

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 *Theobroma cacao* contains Naringenin, Luteolin, Orientin, and Phenyl acetic acid, Phloretic acid etc.It has already been established that *Theobroma cacao* plant belonging to malvaceae family has potential to help controlling malaria. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of malaria.

Enzyme found in *Plasmodium falciparum*

It has been reported that malaria can cause as a result of *Plasmodium falciparum* infestation. Various metabolic cycles have been seen 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 falciparum.* It has been found that L-lactate dehydrogenase enzyme (protein database code1T2E) is involved in Lactate fermentation 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 protozoan 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 *Theobroma cacao* 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 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 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 Glycosmispentaphylla (Retz.) Correa, 2019, 56(2), 111-121).Table 1 shows that L-lactate dehydrogenase phlorectic acid interaction has the highest positive value of -CDOCKER energy 26.2788 and minimum value of the difference 0.6436 between - C DOCKER interaction energy and - C DOCKER energy followed by vanillic acid. Thus, the results indicated that Phloretic acid and vanillic acidcan effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Plasmodium falciparum.* Higher positive values forphloretic acid indicated that it was the most active ingredient against *Plasmodium falciparum.* On the other hand, caffeinecan deactivate the enzyme to a small extent (negative -CDocker energy but positive –CDocker interaction energy). Procyanidin cannot interact with L-lactate dehydrogenase enzyme. Thus, the key phytochemicals preventing malaria caused by *Plasmodium falciparum* are Phloretic acid, Vanillic acid, chlorogenic acid, theobromine.

CONCLUSIONS

From the study it was found that *Theobroma cacao* plant has medicinal action against Malaria. Malaria is caused by *Plasmodium falciparum.* This study was accomplished in order to provide the theoretical basis of this observation. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (like procyanidin, vanillic acid, Theobromine, Phloretic acid, chlorogenic acid, caffeine), which can have a significant interaction with the vital enzyme L-lactate dehydrogenase of the microbe. It was found that phloretic acid, vanillic acid, chlorogenic acid, Theobrominecan form strong bond with the enzyme successfully inhibiting the metabolic cycle of themicrobe. Caffeine was not much effective in deactivating the enzyme of the microbe. Procyanidine cannot deactivate the enzyme. Thus, this study could explain that the presence of phloretic acid, vanillic acid, Theobromine, chlorogenic acid provided the medicinal values to *Theobroma cacao* against malaria caused by *Plasmodium falciparum.*

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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	Chlorogenic acid	16.49	32.7658	16.2758
2	Phloretic acid	26.2788	25.6352	0.6436
3	Theobromine	17.3763	23.462	6.0857
4	Vanillic acid	21.7382	23.2139	1.4757
5	Caffeine	-12.3752	13.8192	26.1944
6	Procyanidine	Failed	Failed	NA

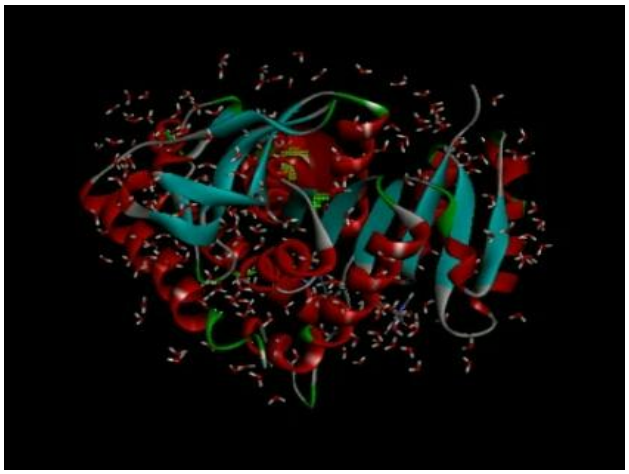

Figure1. Active site of L-lactate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Tinospora cordifolia* against *Neisseria gonorrhoeae* Causing Gonorrhea

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ABSTRACT

Gonorrhea is a sexually transmitted disease. It is most common in young adults. Treating gonorrhea is becoming a challenge due to more drug-resistant strains are evolving. Phytochemicals are non-nutritive compounds obtained from plants. It has been suspected that *Tinospora cordifolia* plant extract is used to cure gonorrhea. The plant extract contains different phytochemicals. Gonorrhea is caused by *Neisseria gonorrhoeae.* The molecular docking of the phytochemicals with the causative microbial 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, Luteolin can effectively deactivate the nitric oxide reductase enzyme there by interrupting the life cycle of *N. gonorrhoeae*.

Keywords: phytochemical, Biovia, Discovery studio, *Tinospora cordifolia*, *Neisseria gonorrhoeae.*

INTRODUCTION

Gonorrhea is a social disease considered as a public nuisance in many developed countries (Barnes and Holmes, 1984). *Neisseria gonorrhoeae* causes infection in the genital tract, oral tract, or rectum. Gonorrhea does not always show symptoms, which is why it is more of a perilous inconvenience. If left untreated it may last for weeks or months with higher risks of complications (Judson, 1990).Development of a potential medications has been complicated by the ongoing evolution of resistant strains and antigenic variation (Baarda and Sikora, 2015). Thus, use of other

contemporary sources other than conventional sources of medication are becoming more of a focus point for researchers. 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 (Chan et al., 2012). 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, 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 et al. 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 while proving to be safe and cost effective. 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 (Benzie and Wachtel-Galor, 2010). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Tinospora cordifolia belongs to family menispermaceae. *T. cordifolia* leaves extract is expected to cure disease like gonorrhea. This plant is known by the common names heart-leaved moonseed, gaduchi, and giloya. It is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of Bangladesh, India, Myanmar, and Sri Lanka (Sinha et al. 2004). *T. cordifolia* is known to contain phytochemicals like Quercetin, Kaempferol, Magnoflorine, Palmatine, Heptacosanol, Luteolin, Choline, Sitosterol, Berberine, Columbin, Syringin, Phenol, Nanocosan etc (Mishra et al. 2014). There is high possibility that these phytochemicals play a major role in curing gonorrhea. However, there is no report identifying the specific phytochemical responsible to cure gonorrhea. *N. gonorrhoeae* are fastidious, Gram-negative cocci that are facultatively intracellular and typically appear in pairs (diplococci), resembling the shape of coffee beans. Gonorrhea is a common sexually transmitted infection (STI). A person can transmit it during any kind of sexual contact. With an early diagnosis, effective treatment is usually available. However, without treatment, gonorrhea can result in long-term complications (Judson, 1990). This study focuses on the identification of the phytochemical of *T. cordifolia* responsible to cure gonorrhea caused by *N. gonorrhoeae.*

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 *T. cordifolia* contains Quercetin, Kaempferol, Magnoflorine, Palmatine,

Heptacosanol, Luteolin, Choline, Sitosterol, Berberine, Columbin, Syringin, Phenol, Nanocosan, etc. It has already been established that *T. cordifolia* plant belonging to Menispermaceae family has potential to help controlling gonorrhea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of gonorrhea.

Enzyme found in *N. gonorrhoeae*

It has been reported that gonorrhea can cause as a result of *N. gonorrhoeae* 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 *N. gonorrhoeae* bacteria. It has been found that nitric oxide reductase enzyme (5GUX) is involved in arginine and proline 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, which 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 *T. cordifolia* plant were downloaded from the website (PubChem). The protein database code of the nitric oxide reductase enzyme was identified from the website 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

Fig. 1 shows the active site of the nitric oxide 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 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 (10).

Table 1 shows that nitric oxide reductase and Luteolin interaction has the highest positive value of -CDOCKER energy (42.8692) and minimum value of the difference (7.2249) between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that Luteolin can effectively deactivate the nitric oxide reductase enzyme thereby interrupting the biological cycle of *N. gonorrhoeae*. Higher positive values for nitric oxide reductase indicated that it was the most active ingredient against *N. gonorrhoeae*. On the other hand, berberine can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Columbin, sitosterol, syringin cannot interact with nitric oxide reductase enzyme. Thus, the key phytochemical preventing gonorrhea caused by *N. gonorrhoeae* is Luteolin.

CONCLUSIONS

It was previously known that *T. cordifolia* plant has medicinal action against gonorrhea. gonorrhea is caused by *N. 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 (Quercetin, Kaempferol, Palmatine, Luteolin, Choline, Sitosterol, Berberine, Columbin, Syringin, Phenol), which can have a significant interaction with the vital enzyme nitric oxide reductase of the microbe. It was found that Luteolin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Berberine is found to be not much effective in deactivating the enzyme of the microbe. Columbin, sitosterol, syringin cannot deactivate the enzyme. Thus, this study could explain that the presence of Luteolin provided the medicinal values to *T. cordifolia* against gonorrhea caused by *N. gonorrhoeae*.

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Table 1. Results of CDocking of phytochemicals with nitric oxide reductase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Luteolin	42.8692	50.0941	7.2249
2	Quercetin	39.4796	42.862	3.3824
3	Kaempferol	37.8529	43.2931	5.4402
4	Phenol	16.8648	19.253	2.3882
5	Choline	8.28563	23.1097	14.82407
6	Palmatine	1.56717	45.8082	44.24103
7	Berberine	-3.8247	46.385	50.2097
8	Sitosterol	F	F	NA
9	Columbin	F	F	NA
10	Syringin	F	F	NA

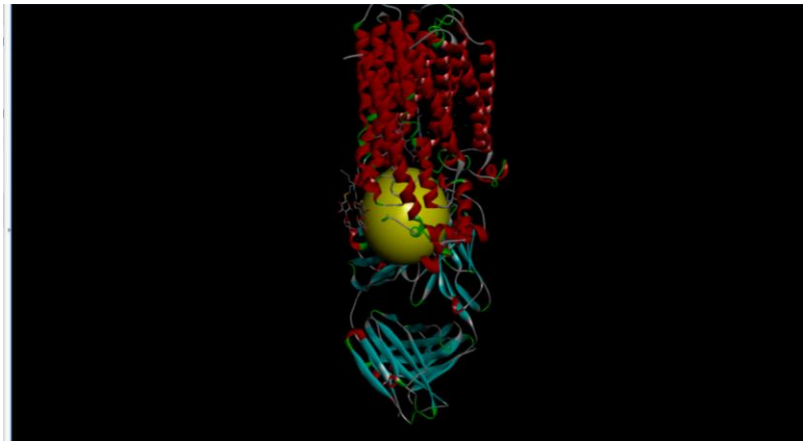

Figure 1. Active site of nitric oxide reductase enzyme

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RESEARCH ARTICLE

In silico screening of Antidiarrheal Phytochemicals from *Coriandrum sativum*

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ABSTRACT

Coriandrum sativum is commonly known as coriander. It belongs to Apiaceae family. It is also known as Chinese parsley. This study indicates that, coriander is also used to cure digestion problems like diarrhea. Studies have shown that, the ethanolic extract of whole plant of *Coriandrum sativum* can be used for its possible analoesic, antidiarrheal, antimicrobial and cytotoxic activity. 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. It has been reported that *Corindrum sativum* plant extract is used to cure diarrhea. The plant extract contains different phytochemicals and from these phytochemicals some are effectively deactivate the *Escherichia coli* which cause the diarrhea.

Key words: Phytochemical, Biovia, Discovery studio, *Coriandrum sativum, Escherichia coli.*

INTRODUCTION

About more year ago, indigenous people traditionally use a wide range of plants to maintain their health.They people were very intelligent about the medicinal plants.As there was no hospitality system in that time and also science was not so developed. So they just believed Ayurveda. Traditional Medicinal plants grow in many areas and some are found only inside the forest. The different parts of the plants (root, bark, leaves, fruit, seed, flower, etc) are collected and their extract is used as medicine [1]. Various plants shows numerous medicinal properties like anti-oxidant, anti-inflamatory, anti-cancer, anti-microbial, anti-diabetes action etc [2]. *Coriandrum sativum* belongs to family Apiaceae. Coriander leaves extract is used to cure disease like diarrhea. *Escherichia coli* is the most common causing agent of diarrhea. Subsequently it has been shown that at least five different pathogenic strain of *E. coli* are used to cause diarrhea. Enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC) produce a non inflammatory diarrhea, whereas enteroinvasive *E.coli* (EIEC), enterohaemorrhagic *E.coli* (EHEC) and

enteroaggregative *E. coli* (EAggEC) produce an inflammatory diarrhea [3]. ETEC is a major cause of diarrhea in infants (up to three episodes per year) and travellers. They produce diarrhea by attaching to the small intestinal mucosa and elaborate one or both of heat labile and heat stable toxins. EPEC attach firmly to the intestinal mucosa leading to dissolution of the brush border by inducing vesiculation of the microvilli [4]. This process is known as attaching-effacement, and in the jejunum and ileum results in a loss of brush border disaccharidase enzymes and a large area of absorptive surface. EPEC are a major cause of summer diarrhea in infants and neonatal diarrhea. EIEC attach to colonic enterocytes, penetrate by an endocytotic mechanism and replicate there in [5].

This results in necrosis and stripping of large areas of colonic mucosa and dysentery similar to but usually less severe than Shigella dysentery. EHEC produce attaching-effacement to the terminal ileal and colonic mucosa and release the toxins, verocytotoxin (VT) 1 or 2. These kill colonic enterocytes and produce haemorrhagic colitis. In addition, VT can damage vascular endothelial cells, leading to haemolytic uraemic syndrome. The role of EHEC in diarrhea in children in the tropics is not known. The most recently described group is the EAggEC. They damage and blunt colonic villi by haemorrhagic necrosis, although the precise pathogenic mechanisms are unclear. EAggEC are a major cause of chronic diarrhea in children [6]. There is high possibility that these phytochemicals play a major role in curing diarrhea. However, there is no report identifying the specific phytochemical responsible to cure diarrhea. A group of *E coli* belonging to family Enterobacteriaceae generally cause diarrhea [7]. Most cases of diarrhea are caused by an infection in the gastrointestinal tract. The microbes responsible for this infection include bacteria, viruses, parasitic organisms. [8]. This study focuses on the identification of the phytochemical of *Coriandrum sativum* 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 *Coriandrum sativum* contains chrysin, hygrine, β-myrcene, γ-terpinene, camphor, β-sitosterol, digoxin, hexacosanoic acid etc. It has already been established that *Coriandrum sativum* plant belonging to Apiaceae family has potential to help controlling diarrhea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of diarrhea.

Enzyme found in *E. coli*

It has been reported that diarrhea can cause as a result of *E. 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 *E. coli.* It has been found that Shikimate dehydrogenase (1NYT) is involved in Phenylalanine, tyrosine and tryptophan biosynthesis metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method was 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 *Coriandrum sativum* plant were downloaded from the website (PubCHEM). The protein database code of the enzyme was identified from the website (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 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 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, b) small difference between -CDOCKER energy and -CDOCKER interaction energy.

Table 1 shows that Shikimate dehydrogenase and quercetin interaction has the highest positive value of -CDOCKER energy (25.3773) and minimum value of the difference (11.1547) between - C DOCKER interaction energy and - C DOCKER energy. Thus this value indicated that quercetin can effectively deactivate the enzyme thereby interrupting the biological cycle of *E. coli.* Higher positive values indicated that, it is the most active ingredient against diarrhea. On the other hand, GAMMA-TERPINENE and Papaverience can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Digoxin, triglyceride &hexacosanoic cannot interact with shikimate dehydrogenase. Thus, the key phytochemicals preventing diarrhea caused by *E. coli* are quercetin and chrysin.

CONCLUSION

It was previously known that *Coriandrum sativum* plant has medicinal action against 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 (quercetin, chrysin, petroselinic acid, hygrine, GAMMA-TERPINENE, papaverience, digoxin, triglyceride, hexacosanoic acid), which can have a significant interaction with the vital enzyme shikimate dehydrogenase of the microbe. It was found that quercetin and chrysin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. GAMMA-TERPINENE and Papaverience were found to be not much effective in deactivating the enzyme of the microbe. Digoxin, triglyceride, hexacosanoic acid cannot deactivate the enzyme. Thus, this study could explain that the presence of quercetin and chrysin provided the medicinal values to *Coriandrum sativum* against diarrhea by *E. coli.*

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Table1. 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	Quercetin	25.3773	36.532	11.1547
2	Chrysin	17.8283	27.4305	9.6022
3	Petroselinic acid	15.3797	33.8343	18.4546
4	hygrine	4.14874	26.9411	22.79236
5	GAMMA-TERPINENE	-29.3942	14.394	43.7882
6	Papaverience	-44.3187	11.0751	55.3938
7	Digoxin	FAILED	FAILED	N
8	Triglyceride	FAILED	FAILED	N
9	Hexacosanoic acid	FAILED	FAILED	N

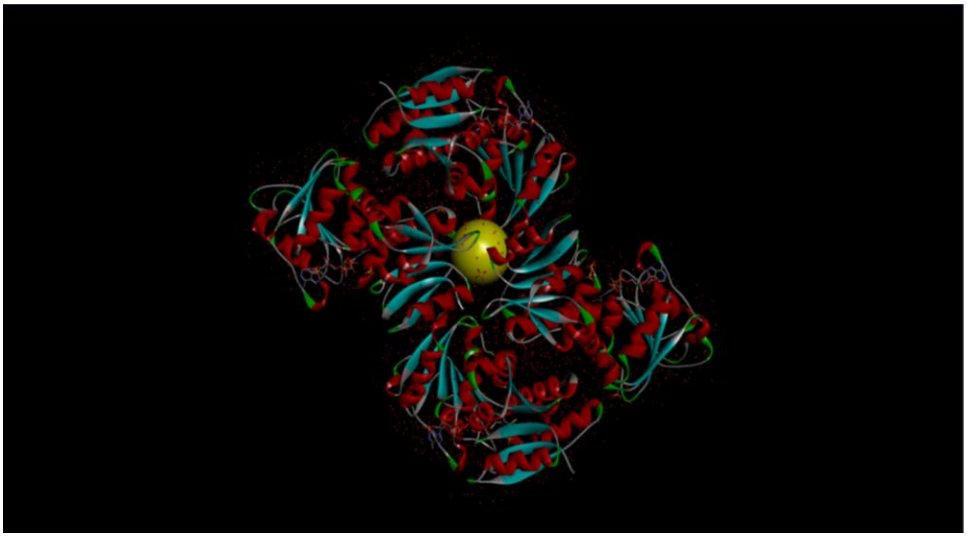

Figure 1. Active site of Shikimate dehydrogenase enzyme.

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals from *Withania somnifera* against Shikimate 5-Dehydrogenase of *Helicobacter pylori* Causing Peptic Ulcer

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ABSTRACT

Withania somnifera (Dunal), popularly known as Ashwagandha, 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 caused by *Helicobacter pylori.* One of the key enzymes involved in its biochemical pathway is shikmate 5-dehydrogenase. The molecular docking of the phytochemicals with the enzyme was 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 the 3 Phyto chemicals, pseudopelletierine can effectively deactivate the shikmate 5-dehydrogenase enzyme thereby interrupting the life cycle of *Helicobacter pylori*.

Keywords: Phytochemicals, Biovia, Discovery studio, *Withania somnifera,* shikmate 5-dehydrogenase, *Helicobacter pylori.*

INTRODUCTION

Nature has been a source of medicinal plants since thousands of years and a number of complementary and alternative drugs have been developed from these sources. The medicinal properties of the plants is due to the phytochemicals, which produce a definite physiological & physicochemical actions in human body. These phytochemicals have therapeutic & diagnostic Functions. Plant based medicinal constituents are derived from some

parts of plants like bark, leaves, flowers, roots, fruits, and seeds (Abdallah, 2011). Phyto-extracts have shown various medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. Medicinal plants play a very important role in human health care & welfare. About 80% of the world population relies on the use of traditional medicines, which is predominantly based on plants & plant based products due to because the herbal products are safe, efficacious, with no side effects, more effective than antibiotics, their cost effectiveness & their feasibility (Abdallah, 2016). Plants play an vital role in production of herbal medicine, alternative medicine, homoeopathy and aromatherapy drugs (Dar et al., 2015). Among different sources of natural & herbal products, plants have been a source of novel chemical substance, which serves as starting materials or raw materials for a number of old and new pharmaceutical products.

Withania somnifera (Dunal), popularly known as Ashwagandha, belongs to family Solanaceae. is a subtropical undershrub commonly used in Indian traditional medicines for more than 3000 years and has been categorized as Rasayana in Ayurveda, which is reported to have defense mechanism against diseases, anti-aging, revitalize the body, increase resistance against adverse environmental conditions to keep mental well-being. The active component of *W. somnifera*, glycowithanolides possesses the ability to alter the cortical and striatal antioxidant enzyme functions (superoxide dismutase, catalase and glutathione peroxidase) in rats. The other biologically active components including alkaloids of ashwagandha, cuscohygrine, anahygrine, topine, etc., steroidal compounds, including ergostane type steroidal lactones, withaferin A, withanolides A–Y, withasomniferin A, withasomnidienone, withasomnierose A–C, withanone, etc. Ashwagandha root powder also exerts free radical scavenging activity (Sankar et al., 2007). Clinical trials and animal research also support the usage of WS for a myriad of conditions such as anxiety, cognitive and neurological disorders, senile dementia, *W. somnifera* also demonstrates neuroprotective effects against 6-OHDA-induced Parkinsonism in rats (Ahmad et al., 2005). Perhaps its antioxidant nature and inhibition of lipid peroxidation in vitro and in vivo is reported to underlie its neuroprotective benefits (Bhatnagar et al., 2009).

Peptic ulcer, also known as PUD or peptic ulcer disease, is an ulcer (defined as mucosal erosions equal to or greater than 0.5 cm) of an area of the gastrointestinal tract that is usually acidic and thus extremely painful (Annonymous, 2007 a, b; 2010). Symptoms includes abdominal pain, classically epigastric with severity relating to mealtimes, after around 3 hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it); bloating and abdominal fullness; waterbrash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus); nausea, and copious vomiting; loss of appetite and weight loss hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus (Roy et al., 2013).

A group of bacteria belonging to genus *Helicobacter pylori* generally cause peptic ulcer. They are spiral shaped Gram negative bacteria. *Helicobacter* infection is a common bacterial disease that affects the Gastro intestinal Tract. *Helicobacter* bacteria typically live in animal and human intestinal tract and are shed through food and water. Humans become infected most frequently through contaminated food and drinking water.This bacteria cause chronic inflammation and colonizes in the antral mucosa layer. This Molecular docking study has been carried out for the identification of the phytochemical of *W. somnifera* responsible to cure peptic 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.

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

Helicobacter sp. causes peptic ulcer. 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 sp.* bacteria. The protein database code of the shikmate 5-Dehydrogenase enzyme was identified from the website RCSB. It has been seen that shikmate 5-Dehydrogenase enzyme (PDB ID-3PHH) is involved in monolignol biosynthesis before oxidative polymerization in the bacterial cell wall (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, pseudopelletierine, which 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. 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 shikmate 5-dehydrogenase enzyme has been presented in Figure 1. 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. It has been indicated that the interaction has the highest positive value of -CDOCKER energy, 0.98 and minimum value of the difference, 19.66 between - C DOCKER interaction energy and - C DOCKER energy (Table 1). Thus the results indicated that pseudopelletierine can effectively deactivate the shikmate 5-dehydrogenase enzyme thereby interrupting the biological cycle of *Helicobacter sp.*. in comparision to the other phytochemicals such as Anahygrine and Cuscohygrine.

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, pseudopelletierine have an effective interaction with the vital enzyme, shikmate 5-dehydrogenase enzyme of the *Helicobacter* sp. It was found that pseudopelletierine can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of pseudopelletierine acid provided the medicinal values to *W. somnifera* against Peptic ulcer caused by *Helicobacter* sp.

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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	-2.63	28.45	31.08
2	Pseudopelletierine	0.98	20.64	19.66
3	Cuscohygrine	-13.47	24.79	38.26

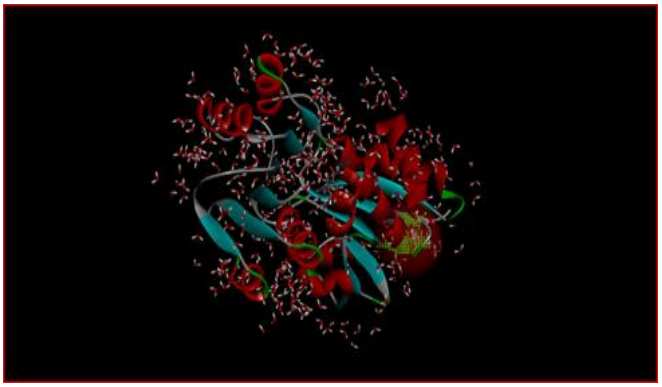

Figure 1. Active site of Shikmate 5-Dehydrogenase enzyme (*H. pylori*)

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Withania somnifera* against 3-Hydroxyacyl-CoA Dehydrogenase Type-2 of *Mycobacterium avium* Causing Pulmonary Infections

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ABSTRACT

This study has been designed to perform the molecular docking of the phytochemicals of *Withania Somnifera* with the enzyme 3-hydroxyacyl-CoA dehydrogenase type-2 from *Mycobacterium avium* causing pulmonary infections in humans with the help of Biovia Discovery Studio. *Mycobacterium avium* belong to a much larger group of bacteria referred to as nontuberculous mycobacteria (NTM), environmental mycobacteria, atypical mycobacteria, or mycobacteria other than tuberculosis. 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, pseudopelletierine and anahygrine can effectively deactivate the 3-hydroxyacyl-CoA dehydrogenase type-2 enzyme thereby interrupting the life cycle of *Mycobacterium avium*.

Key words: phytochemical, Biovia, Discovery studio, *Withania somnifera, Mycobacterium*

INTRODUCTION

Mycobacterium avium complex (MAC) includes at least two species, *Mycobacterium avium* and *Mycobacterium intracellulare*. These organisms belong to a much larger group of bacteria referred to as nontuberculous mycobacteria (NTM), environmental mycobacteria, atypical mycobacteria, or mycobacteria other than tuberculosis (Turenne et al. 2007). Disease manifests as chronic pneumonia; disseminated infection; skin, soft tissue, and bone infection; and acute hypersensitivity pneumonitis. Unlike tuberculosis, MAC is not transmitted from person to person. It is

ubiquitous in the environment; thus one likely acquires it from various exposures to the environment. Organisms are present in soil, water, biofilms, and aerosols. MAC is resistant to disinfectants used in water treatment centers and can thus be isolated from drinking water. MAC is the most frequent cause of pulmonary infection due to an NTM in the United States (Falkinham, 2002). Pulmonary disease due to MAC manifests as a chronic lung infection, with radiographic changes including bronchiectasis, nodules, and/or cavitary lesions. MAC is primarily a pulmonary pathogen that affects individuals who are immune compromised (eg, from AIDS, hairy cell leukemia, immunosuppressive chemotherapy). In this clinical setting, MAC has been associated with osteomyelitis, tenosynovitis, synovitis, and disseminated disease involving lymph nodes, CNS, liver, spleen, and bone marrow (Marras et al. 2007).

Pathogenesis of this disease is poorly understood; according to the literature, the host immune response is very important. Age and preexisting lung disease are major risk factors for active NTM lung disease. There is an interaction between macrophages, lymphocytes and mycobacteria, which is the focal point in the pathogenesis of MAC infection; this interaction can lead to granuloma formation, successful control of the infection, or clinical disease (Martins et al. 2005). The *Withania somnifera* or Indian winter cherry, has been an important traditional herbal medicine for over 3,000 years (Mishra et al. 2000). *W. Somnifera* is a densely pubescent shrub up to 1 m tall belonging to the family of *Solanaceae*. Its root contains flavonoids and many active ingredients like alkaloids and steroidal lactones, which are commonly called withanolides. The chemical constituents of Ashwagandha include three natural powerful antioxidants, superoxide dismutase, catalase, and glutathione peroxidise. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy (Shenoy et al. 2012).

An Indian study proved that use of adjunct therapy of Ayurvedic medicine with anti-tubercular drugs in the therapeutic management of pulmonary infection. The study was conducted among 99 newly diagnosed pulmonary infection patients from both the sexes aged between 10 and 65 years. The subjects were administered 500 mg of Ashwagandha -2 caps, twice daily and Chyawanprash (as per Indian Pharmacopeia)-10 g, thrice daily for a period of 28 days. The study reported amelioration of symptoms, improvement of body weight, normalization of erythrocyte sedimentation rate, appreciable change in IgA and IgM patterns, and significant increase in bioavailability of isoniazid and pyrazinamide (Samal, 2016). This study was designed for the identification of the effective phytochemical among the anahygrine, pseudopelletierine, cuscohygrine of *Withnia somnifera* responsible to cure pulmonary infections caused by *Mycobacterium avium* complex.

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. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling pulmonary infections caused by *Mycobacterium avium* complex.

Enzyme Used

It has been reported that pulmonary infections can be caused by *M. avium* complex 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 *Mycobacterium sp.* bacteria. It has been found that 3-hydroxyacyl-CoA dehydrogenase type-2 from *M. avium* (protein database code 3PPI) is involved in ethanol 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. The protein database code of the 3-hydroxyacyl-CoA dehydrogenase enzyme was identified from the RCSB 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.

RESULTS AND DISCUSSION

The active site of the 3-hydroxyacyl-CoA dehydrogenase enzyme was represented in Figure 1. 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.

The result of the interaction has been presented in Table 1 and the highest positive value of -CDOCKER energy 7.04 , 2.14 and minimum value of the difference between - C DOCKER interaction energy and - C DOCKER energy followed by 20.2, 27.92 by the phytochemicals pseudopelletierine and anahygrine respectively. Thus the results indicated that pseudopelletierine and anahygrine can effectively deactivate the 3-hydroxyacyl-CoA dehydrogenase type-2 enzyme thereby interrupting the biological cycle of *Mycobacterium sp.* Higher positive values for pseudopelletierine indicated that it was the most active ingredient against *Mycobacterium sp.* On the other hand, cuscohygrine cannot deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). and cannot interact with 3-hydroxyacyl-CoA dehydrogenase type-2 enzyme. Thus, the key phytochemical pseudopelletierine preventing pulmonary infection caused by *M. avium.*

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, pseudopelletierine and anahygrine have an effective interaction with the vital enzyme, 3-hydroxyacyl-CoA dehydrogenase type-2 enzyme of the *M. avium.* It was found that pseudopelletierine can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of pseudopelletierine acid and anahygrine provided the medicinal values to *W. somnifera* against the pulmonary infections caused by *M. avium.*

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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	2.14	30.06	27.92
2	Pseudopelletierine	7.04	27.24	20.2
3	Cuscohygrine	-4.96	34.87	39.83

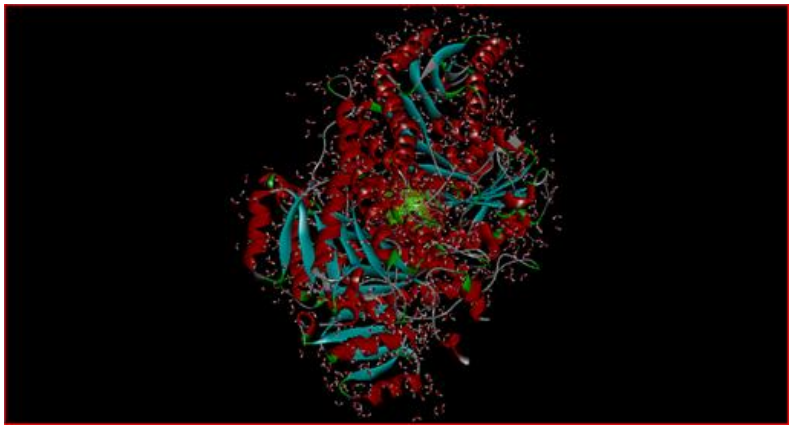

Figure 1. Active site of 3-hydroxyacyl-CoA dehydrogenase type-2 from *Mycobacterium avium*

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RESEARCH ARTICLE

Computer-Aided Investigation of Phytochemicals from *Cuminum cyminum* against *Staphylococcus aureus* based on Molecular Docking Studies

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ABSTRACT

Cuminum cyminum is the source of cumin which have been used for the treatment various healing system in different geographical region. It has been reported that *Cuminum cyminum* plant extract is used to cure boils. The plant extract contains different phytochemicals. Boils is caused by *Staphylococcus sp.* One of the key enzymes involved in its biochemical pathway is pyruvate oxidase. The molecular docking of the phytochemicals with this enzyme was studied by 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 pyruvate Oxidase enzyme there by interrupting the life cycle of *Staphylococcus.*

Key words: phytochemical, Biovia, Discovery studio, *Cuminum cyminum, Staphylococcus*

INTRODUCTION

Phytochemicals are non-nutritive compounds obtained from plants.In ancient time, life was natural, slow, difficult 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. [1]. 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 [2]. The medicinal value of 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. 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]

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. 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. [4]. 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*. [5].

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 [6]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Cumin belongs to family Apiaceae. Cumin extract is used to cure disease like skin boils. Cumin is known to contain phytochemicals like cuminaldehyde, coumarin, 2-ethoxy-3-isopropylpyrazine, anthraquinone, 2-methoxy-3-methylpyrazine etc. [7]. The health benefit of cumin includes its ability to aid in digestion, improve immunity and treats skin disorder. Cumin is also known to relieve respiratory disorder such as asthma and bronchitis [8]. 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 genus *Staphylococcus* generally cause boils. They are round shaped Gram positive bacteria. *Staphylococcus* infection is a common bacterial disease that affects the upper respiratory tract. *Staphylococcus* bacteria typically live in animal and human skin and in upper respiratory tract. Humans become infected most frequently through contaminated water or food. This study focuses on the identification of the phytochemical of *Cuminum cyminum* responsible to cure boils caused by *Staphylococcus 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 *Cuminum cyminum* contains cuminaldehyde, coumarin, 2-ethoxy-3-isopropylpyrazine, anthraquinone, 2-methoxy-3-methylpyrazine, etc.It has already been established that *Cuminum cyminum* plant belonging to *Apiaceae* family has potential to help controlling boils. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of boils.

Enzyme found in *Staphylococcus aureus*

It has been reported that boils can causes a result of *Staphylococcus sp*.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 sp*. It has been found that pyruvate oxidase (protein database code 1EZ9) is involved in pyruvate metabolism (KEGG) and very crucial for survival of the particular microbe.

Molecular docking

Molecular docking method was 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 *Cuminum cyminum* plant were downloaded from the website. The protein database code of the enzyme pyruvate oxidase 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 pyeuvate oxidase 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 pyruvate oxidase-cuminaldehyde interaction has the highest positive value of -CDOCKER energy (19.2293) and minimum value of the difference (1.6156) between - C DOCKER interaction energy and - C DOCKER energy followed by coumarin. Thus the results indicated that cuminaldehyde and coumarin can effectively deactivate the pyruvate oxidase enzyme thereby interrupting the biological cycle of *staphylococcus sp*. Higher positive values for cuminaldehyde indicated that it was the most active ingredient against *Staphylococcus sp*. On the other hand, like2-ethoxy-3-isopropylpyrazine, anthraquinone can deactivate the enzyme to a small extent. Thus, the key phytochemicals preventing boils caused by *staphylococcus sp.* are cuminaldehyde and coumarin.

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, coumarin, anthraquinone, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-methylpyrazine), which have a significant interaction with the vital enzyme (pyruvate oxidase) of the microbe. It was found that chrysin and hygrinecan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. 2-methoxy-3-methylpyrazine,2-ethoxy-3-isopropylpyrazine, anthraquinone, and anthraquinone werefound to be not much effective in deactivating the enzyme of the microbe.Thus, this study could explain that the presence of cuminaldehyde and coumarin provided the medicinal values to *Cuminum cyminum* against boils caused by *Staphylococcus Sp.*

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Table 1. Results of CDocking of phytochemicals with pyruvate oxidase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Coumarin	16.0093	19.2406	3.2367
2	2-methoxy-3-methylpyrazine	7.7575	18.2568	10.4993
3	Cuminaldehyde	19.2293	20.8449	1.6156
4	Anthraquinone	20.2048	25.9412	5.7364
5	2-methoxy-3-isoproylpyrazine	12.8908	22.7523	9.8615

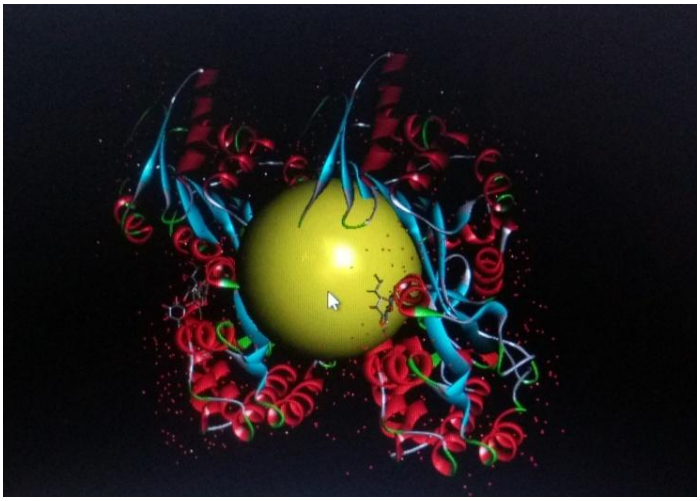

Figure 1. Active site of Pyruvate oxidase enzyme

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RESEARCH ARTICLE

Phytochemical, *In silico* Evaluation on *Chrysopogon zizanioides* (Vetiver) for Treatment of Urinary Tract Infection (UTI)

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ABSTRACT

Urinary tract infection (UTI) is one of the most common and major infectious disease with global expansion. There is a growing concern regarding the resistance of uropathogens towards conventional antibiotics, which leads to therapeutic failures after empiric treatment. Among different representative strains of uropathogens like *Escherichia coli, Klebsiella spp, Proteus, Staphylococcus, Streptococcus* and *Enterococcus,* the most prevalent strain is *E. coli*.Use of phytochemicals for the treatment of infectious diseases is now widely accepted for their efficacy, safety, less side effect and cost effective. *Chrysopogon zizanioides* synonymously known as *Vetiveria zizanioides* (Family: Poaceae/ Gramineae) has a unique characteristic of being xerophytes. It tolerates extreme temperature and grows over a wide range of soil pH. From the ancient time, it is very popular in India for the treatment of skin disorders and used as tonic and blood purifier. Present study focused, the use of Vetiver for the treatment of UTI .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 tannic acid and harmalol can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Escherichia coli.*

Key words: phytochemical, Biovia, Discovery studio, *Chrysopogon zizanioides, Escherichia coli*

INTRODUCTION

Phytochemicals are non-nutritive chemical compounds obtained from plants. In ancient time, life was natural, slow, difficult at times but healthy because of using natural products in their daily life. Today, in modern times, life is fast paced, comfortable, machine dependent, readymade, stressful and unhealthy. Changing work condition, environment, less physical activity, sedentary jobs, comfortable but stressful life and bad food choice has exposed us to some dangerous health problems like blood pressure, diabetes, obesity, stress etc. A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing. Pathogenic micro-organisms causes a number of diseases by their qualities of invasiveness and toxigenesis. Synthetic antibiotics used against them can cause oxidative stress that can lead to damage to DNA, proteins and lipids in human cells, but this effect can be alleviated by antioxidants. Thus as an alternate to this, medicinal plants can be used as they contain phytochemicals and antioxidants that can pave a way for development of antimicrobial drug as well as used in cancer therapy as plants have been used as a source of sophisticated traditional medicines from thousands of years.

Medicinal plants represent a rich source of antimicrobial agents and are widely used either directly as folk remedies or indirectly in the preparation of modern pharmaceutical by all sections of the population. World Health Organization (WHO) noted that more than 80% of the world's population depends on traditional medicine for primary healthcare. Since ages various ailments and infectious diseases have been known to be treated using herbal remedies for the betterment of mankind throughout the world. Thus, scientists are increasingly turning their attention to natural products, either as pure compounds or as standardized plant extracts, looking for new leads to develop better drugs against microbial infections [1]. Grasses from various families are also studied for their potential role in pharmaceutical drugs. The grasses have traditionally been used by the Indian tribes for treating various ailments, diseases and disorders. It has also been used in traditional medicine of Asia and Africa (amritasoni, praveen dahiya, 2015).

Sustainable management of traditional medicinal plant resources is important, not only because of their value as a potential source of new drugs, but due to reliance on traditional medicinal plants for health (A.B. Cunningham,1993) .Vetiver (*Chrysopogon zizanioides*, previously *Vetiveria zizanioides*)is a perennial grass and native to India. Vetiver can grow up to 1.5meters high and form clumps as wide. The stems are tall and theleaves are long, thin, and rather rigid. Vetiver's roots grow downward,2-4 meters in depth [1]. *Vetiveria zizanioides*is profoundly used inAyurvedic medicine. The chemical constituents present in the plantare Vetiverol, Vetivone, Khusimone, Khusimol, Vetivene, Khositone,Terpenes, Benzoic acid,Tripene-4-ol, s-Humulene, Epizizianal, vetivenylvetivenate, iso khusimol, s-vetivone, vetivazulene [2,3]. Ayurvedic literature mentioned that plant is used as digestive, carminative stomachic, constipating, haematinic, expectorant, antispasmodic, antiasthmatic, antigout, anthelmentic, antimicrobical and diuretic (Krishnaveni V,2016).

Crude leaf extract of *Vetiveria zizanioides* showed antibacterial activity against six pathogenic bacteria at 10 mg/ml concentration [4,5]. The methanol leaf extract of vetiver against *Staphylococcus aureus* showed the highest mean zone of inhibition (29mm) was recorded, followed by *Klebsiella pneumoniae* (25 mm), *Escherichia coli* (20mm), *Pseudomonas aeruginosa* (18mm), *Streptococcus faecalis* (17mm) and *Salmonella typhi* (15mm) (D. Sangeetha* and D. Stella,2012). *Escherichia coli* are a very diverse species of bacteria found naturally in the intestinal tract of all humans and many other animal species. A subset of *E. Coli* are capable of causing enteric/diarrhoeal disease, and a different subset cause extra-intestinal disease, including urinary tract infection (UTI) [6].(Carl F. Marrs et al ,2005). This study focuses on the identification of the phytochemical of *Vetiveria zizanioides* responsible for curingurinary tract infection (UTI) caused by *E.coli* [7,8].

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 *Chrysopogon zizanioides* containstannic acid, harmalol, limonene, neoeriocitrin, vetiselinenol etc. It has already been established that *Chrysopogon zizanioides* plant belonging to Apiaceae family has potential to help controlling urinary tract infection (UTI). This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of urinary tract infection (UTI).

Enzyme found in *Escherichia coli*

It has been reported that urinary tract infection (UTI) can cause as a result of *Escherichia coli 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 *Escherichia coli sp.*bacteria. It has been found that shikimate dehydrogenase enzyme (protein database code 1NYT) 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 *Chrysopogon zizanioides* plant were downloaded from the website (refer). The protein database code of the shikimate dehydrogenase enzyme was identified from the website (refer). 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-Anthraquinone interaction has the highest positive value of -CDOCKER energy (8.12) and minimum value of the difference (7.97) between - C DOCKER interaction energy and - C DOCKER energy . Thus the results indicated that Anthraquinone can effectively deactivate the shikimate dehydrogenase enzyme there by interrupting the biological cycle of *Escherichia coli sp.*. Higher positive values for Anthraquinone indicated that it was the most active ingredient against *Escherichia coli sp.* Alpha-vetivones, beta-vetivones and cloven cannot interact with shikimate dehydrogenase enzyme. Thus, the key phytochemicals preventing urinary tract infection (UTI) caused by *Escherichia coli sp.*is Anthraquinone.

CONCLUSION

It was previously known that *Chrysopogon zizanioides* plant has medicinal action against urinary tract infection (UTI). Urinary tract infection (UTI) is caused by *Escherichia coli 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 (Alpha-vetivones, Anthraquinone, Beta-vetivones and cloven), which can have a significant interaction with the vital enzyme (shikimate dehydrogenase) of the microbe. It was found that Anthraquinone can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha-vetivones, beta-vetivones and cloven cannot deactivate the enzyme. Thus, this study could explain that the presence of Anthraquinone provided the medicinal values to *Chrysopogon zizanioides* against urinary tract infection (UTI) caused by *Escherichia coli Sp.*

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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	Alpha-vetivones	-35.76	15.62	51.38
2	Anthraquinone	8.12	16.09	7.97
3	Beta-vetivones	-42.33	22.23	64.56
4	Cloven	-35.29	15.15	50.44

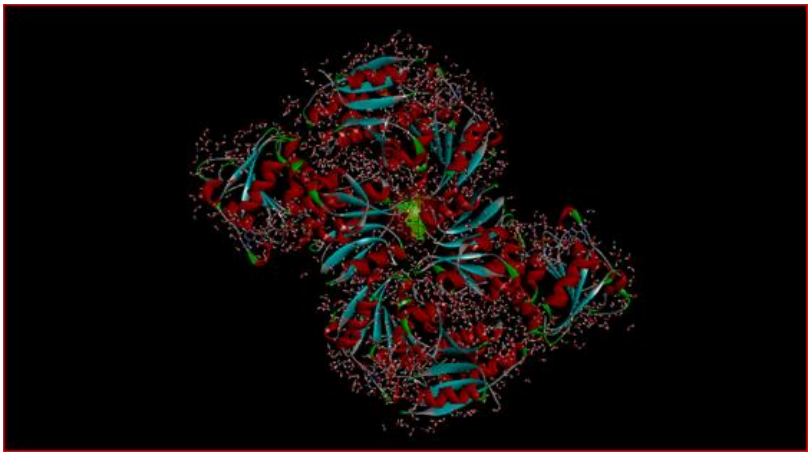

Figure 1. Active site of shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals Screened from *Nardostachys jatamansi* against Acetoacetyl CoA Reductase of Candida tropicalis Causing Candidiasis

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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 candidiasis. The rhizomes of *Nardostachys jatamansi*, the plant commonly known as Jatamansi have been described in Ayurveda for their soothing and sedative action on the central nervous system. The plant extract contains different phytochemicals. Since time immemorial man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. No doubt, plants are serving several purposes whether health, nutrition, beauty or medicinal. With the development in techniques and recent researches, it has been proved that certain nutritive and non-nutritive chemicals in plants which are of very much importance to human diet possess heeling properties. Plants and its phytoconstituents can also be used to treat fungal infections particularly Candidiasis such as oropharyngeal candidiasis, vulvovaginal candidiasis and others such as spirotrichosis, chromoblastomycosis, Tinea pedis etc. Candidiasis is caused by *Candida tropicalis*. One of the key enzymes involved in its biochemical pathway is acetoacetyl CoA 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 -C DOCKER energy and -C DOCKER interaction energy. High positive values for both the parameters indicated that out of different phytochemicals 1-octacosanal can effectively deactivate the enzyme thereby interrupting the life cycle of *Candida tropicalis*.

Key words: phytochemical, BIOVIA, Discovery studio, *Nardostachys jatamansi*, *Candida tropicalis*.

INTRODUCTION

Herbs have been one of the important and unique sources of medicines even since the dawn of human civilization. In spite of tremendous development in the field of allopathy during the 20th century, plants still remain one of the major sources of drug in the modern as well as traditional system of medicine throughout the world. Over 60% of all pharmaceuticals are plant-based. Plants and its phytoconstituents are used not only used to prevent but also used to cure various disorders. Various researches on plants all over the world either have been or have to be carried out for eradicating ailments. Most common of which is fungal infections, especially Mucosal Candidiasis caused by fungi *Candida albicans*. The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. 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 is the best combinational chemist and has cure for all diseases and illness of mankind. Medicinal plants are back bone of several indigenous traditional system of medicine. Traditional used medicinal plants are screened for their antimicrobial efficacy (kalayou et al., 2012). The undesirable effect of the modern medicine has diverted the attention of the people toward herbal medicines. 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 [1]. 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 [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]. 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. [4] Natural antioxidants occur in all higher plants and in all parts of the plant like wood, bark, stems, pods, leaves, fruits, roots, flowers and seeds (Kim et al., 1997). It is now well recognized that the antioxidant activity of these plants is mainly due to the presence of phenolic compounds (Knezeric et al., 2012).

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. [5]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Jatamansi belongs to family Caprifoliaceae. Methanolic extract is used to cure disease like candidiasis. Jatamansi is known to contain phytochemicals like 1-octancosanol, beta-sitosterol, oleanolic acid, ursolic acid etc. Candia normally lies on the skin and inside the body, in place such as the mouth, throat, gut and vagina without causing any problem. Oral thrush, candidiasis that develops in mouth or throat is called thrush. Tiredness and fatigue, recurring genital or urinary tract infections, digestive issue, Sinus infection, joint pains are some of the most common symptoms for the infection.

There is high possibility that these phytochemicals play a major role in curing candidiasis. However, there is no report identifying the specific phytochemical responsible to cure candidiasis.

A group of yeast/ fungus belonging to family *Saccharomycetaceae* generally cause candidiasis. *Candida* appears as gram positive. Budding yeast cell (blastoconidia) and/or pseudo hyphae showing regular points of construction. This study focuses on the identification of the phytochemical of *Nardostachy jatamansi* responsible to cure candidiasis caused by *Candida tropicalis* yeast (a type of fungus).

MATERIALS AND METHOD

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 *Nardostachy jatamansi* contains 1-octacosanol, beta-sitosterol, oleanolic acid, ursolic acid, etc. It has already been established that plant *Nardostachy jatamansi* belonging to Caprifoliaceae family has potential to help controlling candidiasis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling candidiasis.

Enzyme found in *Candida albicans*

It has been reported that candidiasis can cause as a result of *Candida tropicalis* 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 candida tropicalis. It has been found that acetoacetyl CoA reductase (protein database code 3GK3) is involved in glyoxylate, dicarboxylate 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 *Nadrostachys jatamansi* plant were downloaded from the website [6]. The protein database code of 3GK3 the enzyme was identified from the website [7]. 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.

RESULT AND DISCUSSION

Fig. 1 shows the active site of 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. -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 [8]. Table 1 shows the acetoacetyl-CoA reductase – 1-octacosanol interaction has the highest positive value of -C DOCKER energy (64.0539) and minimum value of the difference (11.388) between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that 1-octacosanol can effectively deactivate the enzyme acetoacetyl-CoA reductase there by interrupting the biological cycle of candida tropicalis. Higher positive values for indicated that it was the most active ingredient against candida tropicalis. On the other hand beta-sitosterol, oleanolic acid, urasolic acid, hinokinin can deactivate the enzyme to a small extent (negative -C DOCKER energy but positive -C DOCKER interaction energy). Thus, the key phytochemicals preventing candidiasis caused by candida tropicalis are acetoacetyl CoA reductase.

CONCLUSION

It was previously known that *Nardostachy jatamansi* plant has medicinal action against candidiasis. Candidiasis is caused by candida tropicalis. 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, beta-sitosterol, oleanolic acid, ursolic acid, which can have a significant interaction with the vital enzyme acetoacetyl-CoA reductase of the microbe. It was found that 1-octacosanal can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Beta-sitosterol, Oleanolic acid, urasolic acid, hinokinin, were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of 1-octacosanol provided the medicinal values to against *Nardostachy jatamnsi* caused by candida tropicalis,

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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	1-octacosanol	64.0539	75.4419	11.388
2	Beta-sitosterol	-40.0336	51.6158	91.6494
3	Oleanolic acid	-140.088	21.3579	161.4459
4	Urasolic acid	-134.109	18.2213	152.3303
5	Hinokinin	-0. O920676	47.1681	47.2601676

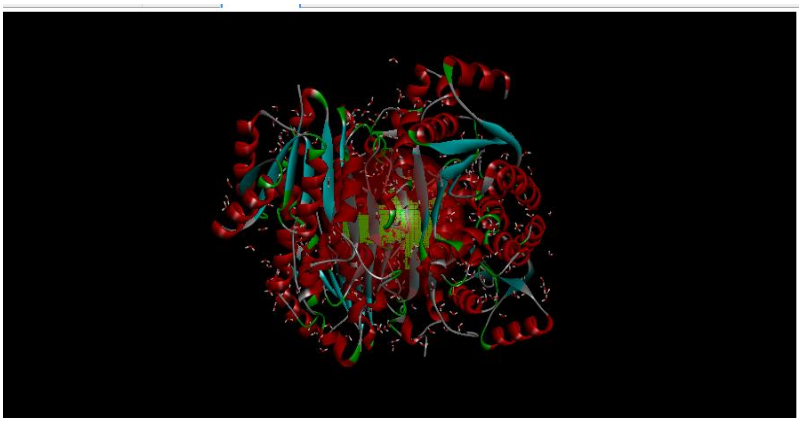

Figure 1. Active site of acetoacetyl CoA reductase enzyme.

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals Screened from *Tagetes erecta* against Isocitrate Dehydrogenase Enzyme of *Staphylococcus aureus* Causing Conjunctivitis

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Tagetes erecta* plants extract is used to cure conjunctivitis. The plant extract contains different phytochemicals. conjunctivitis is caused by *Staphylococcus aureus.* One of the key enzymes involved in its biochemical pathway is isocitrate 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 -C Docker energy and -C Docker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals Isorhamnetin and can effectively deactivate the isocitrate dehydrogenase enzyme thereby interrupting the life cycle of the disease. The flowers are especially employed to cure eye diseases, colds, conjunctivitis, coughs, ulcer, bleeding piles and to purify blood. The extractive efficiency of phenolic compounds from plant material is greatly dependent on the choice of solvent. In this study, six solvents of different polarity viz., hexane, toluene, ethyl acetate, acetone, methanol and water have been used for the extraction of *Tagetes erecta L.* flower by individual cold percolation method.

Key words: phytochemical, BIOVIA, Discovery studio, *Tagetes erecta, Staphylococcus aureus.*

INTRODUCTION

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 [1]. 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 is the best combinational chemist and has cure for all diseases and illness of mankind. Medicinal plants are back bone of several indigenous traditional system of medicine. Traditional used medicinal plants are screened for their antimicrobial efficacy. The undesirable effect of the modern medicine has diverted the attention of the people toward herbal medicines. 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 [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]. 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. [4]. 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. [5].

Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Medicinal plants produce many substances that are biologically active and working together catalytically and synergistically to increase the activity. The plants have effective capacity to scavenge free radicals and represent a source of multifunctional properties. Medicinal plants are rich in secondary metabolites that exhibit a remarkable diversity of both chemical structures and biological activities and are promising source of lead compounds for new drugs targeting neurodegenerative diseases. Marigold belongs to family Asteraceae. Marigold leaves extract is used to cure disease like conjunctivitis. Marigold is known to contain phytochemicals likelupeol, isoquercetinquercetin, alpha-piene, di (Hydroxyethyl) ether, isorhamnetin, stigma sterol, oleanolicacid, alpha-thujene,brainpi (4,5) P2, beta-sitosterol, sabinene, loliolide, ethyl glucuronide, erythrodiol, etc.

There is high possibility that these phytochemicals play a major role in curing conjunctivitis. However, there is no report identifying the specific phytochemical responsible to cure conjunctivitis. *Tagetes erecta L.* popularly known as marigold, is grown as an ornamental plant. Flowers of this plant are used in garlands for societal and religious purposes in Eastern countries. The flowers are usually thrown away after their spiritual uses. This plant belongs to the family Asteraceae (Compositae). Different parts of *T. erecta* plant including flower is used in folk medicine. The flowers are especially employed to cure eye diseases, colds, conjunctivitis, coughs, ulcer, bleeding piles and to purify blood (Manjunath 1969; Kirtikar et al. 1994; Ghani, 2003). The group of marigold plant belongs to the family Asteraceae cause conjunctivitis. It can be caused by allergies or a bacterial or viral infection. Conjunctivitis or pink eye is an irritation or inflammation of the conjunctiva, which covers the white part of the eyeball. Conjunctivitis can be extremely contagious and is spread by contact with eye secretions from someone who is infected. It spreads easily.

Treatable by a medical professional, usually self- diagnosable, lab tests or imaging rarely required, shortterm:resoles within days to weeks.This study focuses on the identification of the phytochemical of *Tagetes erecta* responsible to cure conjunctivitis 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* contain phytochemicals like Lupeol, isoquercetin, alpha-piene, di(Hydroxyethyl) ether, isorhamnetin, stigma sterol, oleanolicacid, alpha-thujene, brainpi (4,5) P2, beta-sitosterol, sabinene, loliolide, ethyl glucuronide, erythrodiol, etc. It has already been established that *Tagetes erecta* plant belonging to *Asteraceae* family has potential to help controlling conjunctivitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of conjunctivitis.

Enzyme found in *Staphylococcus aureus*

It has been reported that conjunctivitis can cause as a result of *Staphylococcus aureus* 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 isocitrate dehydrogenase (protein database code2B0T) is involved in ((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* plant were downloaded from the website [6]. The protein database code of the 2B0T enzyme was identified from the website [7]. 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 BIOVIA software 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.

RESULT AND DISCUSSION

Fig. 1 shows the active site of the Isocitrate 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. -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. [8]. Table 1 shows that isocitrate dehydrogenase - Isorhamnetin interaction has the highest positive value of -C DOCKER energy 21.2897 and 26.8774 value of the difference 48.1671 between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that isorhamnetin can effectively deactivate the isocitrate dehydrogenase enzyme thereby interrupting the biological cycle of *Staphylococcus aureus.* Higher positive values for indicated that it was the most active ingredient against *Staphylococcus aureus.* On the other hand alpha-piene, loliolide, erithrodiol, lupeol, oleanolic acid, brain pi(4,5)P2 can deactivate the enzyme to a small extent (negative -C Docker energy but positive -C Docker interaction energy). Brain pi (4,5) P2 acid cannot interact with isocitrate dehydrogenase. Thus, the key phytochemicals preventing conjunctivitis caused by *Staphylococcus aureus* are isocitrate dehydrogenase.

CONCLUSION

It was previously known that *Tagetes erecta* plant has medicinal action against conjunctivitis. Conjunctivitis 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 (lupeol, isoquercetin, quercetin, alpha-piene, Dl(Hydroxyethyl)ether, isorhamnetin, stigma sterol, oleanolic acid, alpha-thujene, brain pi(4,5)P2, beta-sitosterol, sabiene, loliolide, ethyl glucuronide, erythrodiol), which can have a significant interaction with the vital enzyme isocitrate dehydrogenase of the microbe. It was found the Isorhamnetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. (alpha-piene, loliolide, erithrodiol, lupeol, oleanolic acid, were found to be not much effective in deactivating the enzyme of the microbe. Brain pi (4,5) P2 cannot deactivate the enzyme. Thus, this study could explain that the presence of isorhamnetin provided the medicinal values to *Tagetes erecta* against conjunctivitis caused by *Staphylococcus aureus.*

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

SL.NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	Isoharmnetin	21.2897	26.8774	5.5877
2	Alpha-piene	-11.1846	13.884	25.0686
3	Loliolide	-13.6652	17.2795	30.9447
4	Erithrodiol	-70.5985	28.073	98.6715
5	Lupeol	-81.207	21.8328	103.0398
6	Oleanolic acid	-71.3839	22.899	94.2829
7	Brain pi(4,5)P2.sdf	Failed	Failed	Failed

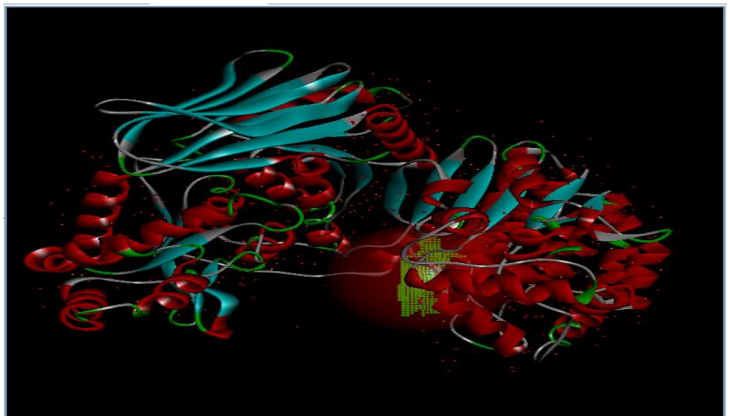

Figure 1. Active site of isocitrate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Molecular Docking Studies of Phytochemicals Screened from *Curcuma longa* (Turmeric) against Homoserine Dehydrogenase of *Staphylococcus aureus* Causing Eczema

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants through primary or secondary metabolism.Wound healing is a complex process of recovering the forms and functions of injured tissues. The process is tightly regulated by multiple growth factors and cytokines released at the wound site. Any alterations that disrupt the healing processes would worsen the tissue damage and prolong repair process. Various conditions may contribute to impaired wound healing, including infections, underlying diseases andmedications. Numerous studies on the potential of natural products with anti-inflammatory, antioxidant, antibacterial and pro-collagen synthesis properties as wound healing agents have been performed. Their medicinal properties can be contributed by the content of bioactive phytochemical constituents such as alkaloids, essential oils, flavonoids, tannins, saponins, and phenolic compounds in the natural products. The plant extract contains different phytochemicals. It has been reported that *Curcuma longa* plant extract is used to cure Eczema. Eczema is caused by *Staphylococcus aureus* one of the key enzymes involved in its biochemical pathway is peroxiredoxin. Turmeric (*Curcuma longa*), a commonly used spice throughout the world, has been shown to exhibit anti-inflammatory, antimicrobial, antioxidant, and anti-neoplastic properties. 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 Demethoxycurcumin and flavonoids can effectively deactivate the Homoserine Dehydrogenase thereby interrupting the life cycle of Staphylococcus.

Key words: phytochemical, Biovia, Discovery studio, *Curcuma longa, Staphylococcus*

INTRODUCTION

In former days, life was natural, steady, difficult at times but healthy. Now, in modern times, life is fast paced, comfortable, readymade, stressful and unhealthy. Changing work condition, environment, less physical activity, sedentary jobs, comfortable but stressful life and bad food habits has exposed us to some dangerous health problems 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. Throughout time, the use of plants and plant-derived products have and continue to offer a plethora of health benefits. Many medicinal plant species worldwide are used in traditional medicine for treating different diseases. The world health organization (WHO) has estimated that about 80% of the population living in the developing countries depends tremendously on traditional medicine for their primary health needs. More than half of the world's population still depends exclusively on medicinal plants, and plants offer the active ingredients of most traditional medical products (Kumar and Navaratnam, 2013).

Human skin is the largest organ in the body. It forms the first guard line. Its three main layers are epidermis, dermis and hypodermis (subcutaneous tissue). Each layer offers a distinctive role in the homeostasis of the skin. In addition to rich culinary uses, spices in particular, contribute striking health benefits (Newman *et al*., 2003). Some herbs and spices are known to exhibit anti-cancerous and antioxidant activities (Tapsell *et al*., 2006). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Aggarwal BB et al 2007). Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Various medicinal plants have been used for years in daily life to treat disease all over the world (Ammon HPT and Wahl MA ,1991).

They have been used as a source of potent and powerful drugs (Bansod S, Rai M. 2008). There has been a revival of interest in herbal medicines. The use of medicinal plants for the treatment of many diseases is associated to folk medicine from different parts of the world. Natural products from some plants, fungi, bacteria and other organisms, continue to be used in pharmaceutical preparations either as pure compounds or as extracts. There is a great variety of compounds that can be extracted and characterized from plants (CAC Araújo,2001) India has a rich history of using plants for medicinal purposes. Turmeric (*Curcuma longa* L.) is a medicinal plantextensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases. It is used as a main ingredient of cooking in Asian countries. Because of its yellow colour it is also employed as a dye. It has been widely investigated and found to have many applications. Important applications are in cancer, diabetes, asthma, anemia and intestinal disorders. In dermatology, it has wonderful wound healing activity. Furthermore, it improves skin complexion. It has antioxidant, anti-inflammatory, antiviral, antibacterial and antiseptic properties (Satoskar et al., 1986; Ramirez-Bosca et al., 1995; Khiljee et al., 2011) *C. longa L.*,botanically related to ginger belongs to the Zingiberaceae family [8] is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish-yellow incolour.

Turmeric rhizome is used as a food additive (spice), preservative and colouring agent [9] in Asian countries, including China and South East Asia. It is also considered as auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. Various sesquiterpenes and curcuminoids have

beenisolated from the rhizome of *C. longa*, attributing a wide array of biological activities such as antioxidant [10] anti-inflammatory [11] wound healing [12], anticancer [13] and antibacterial activity [14] (R. Arutselvi et al, 2012) One of the inflammation-based diseases is atopic dermatitis (atopic eczema), which is a chronic disease affecting people genetically tended to overreact to external factors. It is commonly found in association with allergic rhinitis, asthma, or other manifestations of atopy. Atopic dermatitis is a wide spread dermatologic disease in children. The most commonly observed manifestations of atopic dermatitis are extreme skin dryness and itching, redness, scaly patches, and thickened lichenified plaques with excoriation. *Staphylococcus aureus* is being noticed to inhabit skin. Many herbs are used to treat various skin diseases including eczema. *Curcuma longa* and *Matricaria chamomilla* used for the treatment of eczema. (Shadi T. Zari 1 and Talal A. Zari, 2015)

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 have reported *Curcuma longa* to contain Demethoxycurcumin, flavonoids, terpenoids, tannis, bisdemethoxycurcumin, phlobatannis, and coumarin etc. It has already been established that *Curcuma longa*plant belonging to family Zingiberaceae 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 *Streptococcus aureus*

It has been reported that Eczema can cause as a result of *Staphylococcus* 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 *Staphylococcus sp.* bacteria. It has been found that peroxiredoxin enzyme (protein database code 4PG5) is involved in some metabolism 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 *Curcuma longa* plant were downloaded from the website (refer). The protein database code of the alchol dehydrogenase enzyme was identified from the website (refer). 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 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. -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 peroxiredoxin-Phlobatannis interaction has the highest positive value of -C DOCKER energy (10.49) and minimum value of the difference (1.67) between - C DOCKER interaction energy and - C DOCKER energy followed by flavinoids. Thus, the results indicated that Phlobatannis, Desmethoxycurcumin, coumarin, tannis and flavinoids can effectively deactivate the peroxiredoxin enzyme thereby interrupting the biological cycle of *Staphylococcus aureus sp.* Higher positive values for Phlobatannis indicated that it was the most active ingredient against *Staphylococcus aureus sp*. On the other hand, demethoxycurcumin, coumarin, tannis and flavinoids can deactivate the enzyme to a small extent (negative -C DOCKER energy but positive -C DOCKER interaction energy). Terpinoids cannot interact with peroxiredoxin enzyme. Thus, the key phytochemicals preventing diarrhea caused by *Staphylococcus aureus sp.* are Phlobatannis and Demethoxycurcumin.

CONCLUSION

It was previously known that *Curcuma longa* plant has medicinal action against Eczema. Eczema 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 (Demethoxycurcumin, flavonoids, tannis, bisdemethoxycurcumin, phlobatannis and coumarin), which can have a significant interaction with the vital enzyme (peroxiredoxin) of the microbe. It was found that Phlobatannis, Demethoxycurcumin, coumarin, tannis and flavonoids can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Bisdemethoxycoumarin was found to be not much effective in deactivating the enzyme of the microbe. Terpinoids cannot deactivate the enzyme. Thus, this study could explain that the presence of Phlobatannis, Demethoxycurcumin, Coumarin, tannis and flavinoids provided the medicinal values to *Curcuma longa* against diarrhea caused by *Staphylococcus aureus Sp*.

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Table 1. Results of CDocking of phytochemicals with peroxiredoxin(receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Demethoxycurcumin	9.6	11.83	2.23
2	bisdemethoxycurcumin	31.28	41.35	10.07
3	Flavonoids	22.03	29.02	6.99
4	Tannis	29.03	25.10	3.93
5	Phlobatannis	10.49	12.16	1.67
6	Terpinoids	-59.09	26.37	85.46
7	coumarin	17.27	20.82	3.55

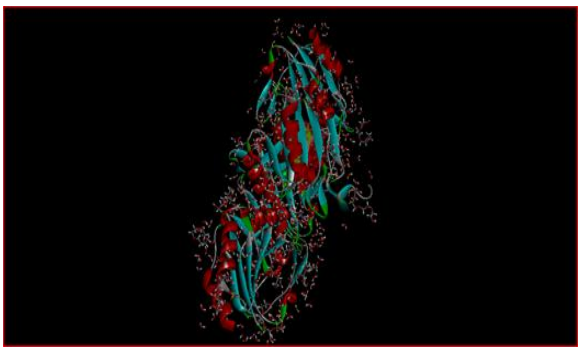

Figure 1. Active site of Homoserine Dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Foeniculum vulgare* against Alkaline Phosphatase of *Prevotella intermedia*

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Foeniculum vulgare* plant extract is used to cureoral ulcer. The plant extract contains different phytochemicals. Oral ulcer caused by *Prevotella intermedia.* One of the key enzymes involved in its biochemical pathway is alkalinephosphatase. 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 caprylic acid and caproic acid can effectively deactivate the alkalinephosphatase enzyme thereby interrupting the life cycle of *Prevotella intermedia*.

Key words: Phytochemical, Biovia, Discovery studio, *Foeniculum vulgare, Prevotella intermedia*

INTRODUCTION

Oral ulceration is a common complaint of patients attending out-patient department. The estimated point prevalence of oral ulcers worldwide is 4%, with aphthous ulcers being the most common, affecting as many as 25% of the population worldwide.[1,2] Oral ulcer (or mouth ulcers) can be very painful and the resulting lesions can be mild or severe. Although oral ulcer has been the subject of considerable studies, the etiology and patho- genesis of the disease have not yet been completely explained [3]. Local and systemic conditions, genetic, immunological and infectious factors have been identified as potential etiological agents. 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 [4]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc.[5]. 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 [6]. It has been estimated that up to 50% of the prescription dispensed in USA may contain one or more natural product drug.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.

The WHO has emphasized the utilization of indigenous system of medicine based on the locally available raw material, i.e. medicinal plants. Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. Medicinal plants play a key role in human health care. 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.

Fennel (*Foeniculum vulgare*) is a flowering plant in the family Apiaceae. It is a traditional and popular herb with a long history of use as a medicine. A series of studies showed that *F. vulgare* effectively controls numerous infectious disorders of bacterial, fungal, viral, mycobacterium, and protozoal origin [7,8,]. An extract of Fennel seeds isused to cure disease like oral ulcer.Fennel is used by mouth for various digestive problems including heartburn, intestinal gas, bloating, loss of appetite, and colic in infants among others.It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and oestrogenic activities. [9,10]. Fennel known to contain phytochemicals rosmarinic acid, d-limonene, caproic acid, lauricacid, oleic acid, myristic acid, caprylic acid [7]. There is high possibility that these phytochemicals play a major role in cure of oral ulcer. However, there is no report identifying the specific phytochemicals responsible to cure oral ulcer.

Prevotella intermedia is a gram-negative, obligate anaerobic pathogenic bacterium involved in periodontal infections, including gingivitis and periodontitis, and often found in acute necrotizing ulcerative gingivitis. It is commonly isolated fromdental abscesses, where obligate anaerobes predominate. *P. intermedia* is also classified as a black pigmented bacteria because of its formation of shiny and smooth colonies which appear either a grey, light brown, or black color on blood agar plates [12]. *P. intermedia* have been found to possess exopolysaccharides which are composed of neutral sugars and mannose and are a major component of biofilm formation. These exopolysaccharides provide *P. intermedia* the ability to evade the innate human immune system [13].*P. intermedia* metabolites are known to initiate and promote oral disease, both directly and indirectly [14].

An oral ulcer is an ulcer that occurs on the mucous membrane of the oral cavity [15]. An oral ulcer is a break in the skin or mucous membrane with loss of surface tissue and the disintegration and necrosis of epithelial tissue [16].The two most common causes of oral ulceration are local trauma and aphthous stomatitis, a condition characterized by recurrent formation of oral ulcers. An ulcer is a tissue defect which has penetrated the epithelial-connective tissue border, with its base at a deep level in the submucosa, or even within muscle or periosteum [17]. An ulcer is a deeper breach of epithelium compared to an erosion or excoriation, and involves damage to both epithelium and lamina propria [18]. These present clinically as multiple, small, round, or ovoid ulcers, with circumscribed margins, covered by a yellowish or gray-white fibrinous exudate and surrounded by an erythematous halo, and present first in childhood or adolescence [19]. Most mouth ulcers are harmless and resolve by themselves within 10 days. Other types of mouth ulcers, such as the aphthous variety or those caused by herpes simplex infection, need topical treatment such as a mouthwash, ointment or gel. This study focuses on the identification of the phytochemical of *Foeniculum vulgare* responsible to cure oral ulcer caused by *Prevotella intermedia.*

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 *Foeniculum vulgare* contains rosmarinic acid, d-limonene, caproic acid, lauric acid, oleic acid, myristic acid, caprylic acid. It has already been established that *Foeniculum vulgare* plant belonging to Apiaceae family has potential to help controlling oral ulcer. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of oral ulcer.

Enzyme Found in *Prevotella intermedia*

It has been reported that oral ulcercan cause as a result of *Prevotella intermedia* 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 *Prevotella intermedia* bacteria. It has been found thatalkaline phosphatise (protein database code1EW9) is involved in sulfopterin metabolism (BRENDA) [20] and is very essential for survival of the particular microbe.

Molecular Ducking

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 *Foeniculum vulgare* plant were downloaded from the website [21]. The protein database code of the alkaline phosphatase enzyme was identified from the website [22]. 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 alkaline phosphatase 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 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) [23].

Table 1 show that alkaline phosphatase-caprylic acid interaction has the highest positive value of -CDOCKER energy (18.9596) and minimum value of the difference (0.6986) between - C DOCKER interaction energy and - C DOCKER energy followed by caproic acid. Thus the results indicated that caprylic acid and caproic acid can effectively deactivate the alkaline phosphatase enzyme thereby interrupting the biological cycle of *Prevotella intermedia*. Higher positive values for caprylic acid indicated that it was the most active ingredient against *Prevotella intermedia*. On the other hand rosmarinic acid, d-limonene, lauric acid, oleic acid, myristic acid cannot interact with alkaline phosphatase enzyme. Thus, the key phytochemicals preventing cholera caused by *Prevotella intermedia* are caprylic acid and caproicacid.

CONCLUSION

It was previously known that *Foeniculum vulgare* plant has medicinal action against oral ulcer. Oral ulcer is caused by *Prevotella intermedia.* 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 (rosmarinic acid, d-limonene, caproic acid, lauric acid, oleic acid, myristic acid, caprylic acid), which can have a significant interaction with the vital enzyme alkaline phosphatase of the microbe.. It was found that caprylic acid and caproic acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Rosmarinic acid, d- limonene, lauric acid, oleic acid, myristic acid, cannot deactivate the enzyme. Thus, this study could explain that the presence of caprylicacid and caproicacid provided the medicinal values to *Foeniculum vulgare* against oral ulcer caused by *Prevotella intermedia*.

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Table 1. Results Of Cdocking Of Phytochemicals With Alkaline Phosphatase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between - C DOCKER interaction energy and - C DOCKER energy
1	Caproic acid	17.1601	15.1712	1.9889
2	Caprylic acid	18.9596	18.261	0.6986
3	d-limonene	Failed	Failed	NA
4	Lauric acid	Failed	Failed	NA
5	Myristic acid	Failed	Failed	NA
6	Oleic acid	Failed	Failed	NA
7	Rosmarinic acid	Failed	Failed	NA

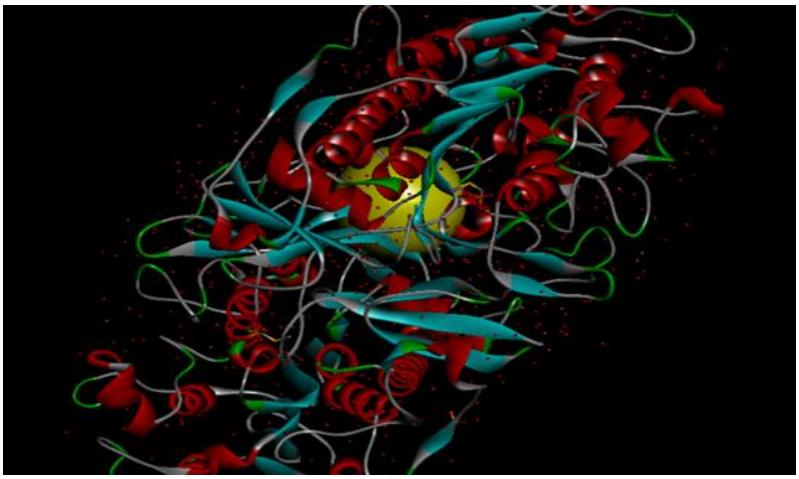

Fig. 1. Image of Receptor Ligand Interaction

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RESEARCH ARTICLE

In silico Analysis of Effects of Phytochemicals from *Linum usitatissimum* against *Helicobacter pylori* Causing Peptic Ulcer

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ABSTRACT

Diet and lifestyle are major factors thought to influence susceptibility to many diseases. Peptic ulcer is also related to the hectic lifestyles although it is caused by bacteria. Phytochemicals are non-nutritive compounds or chemical compounds that are obtained from plants. It has been reported that *Linum usitatissimum* plant extract is used to cure peptic ulcer. The plant extract contains different phytochemicals. Peptic ulcer is caused by *Helicobacter pylori.* The molecular docking of the phytochemicals with the microbial 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 P-coumaric acidcan effectively deactivate the microbial metabolic enzyme thereby interrupting the life cycle of *Helicobacter pylori* .

Key Words: Phytochemical, Biovia, Discovery studio, *Linum usitatissimum, Helicobacter pylori.*

INTRODUCTION

In ancient days, life was very 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 can prevent our life from these dangerous hazards. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from different phytochemicals of plants. For thousands of years human has been

using plant and their product for the treatment of numerous aliments. The traditional or the folk medicines comprises of knowledge and recently the internet in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide.

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 [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. [2]. Medicinal plants play a important role in human health care.

Flaxseed belongs to family Linaceae. It is a food and fiber crop cultivated in cooler regions of the world. Flax is grown for its seeds, which can be ground into a meal or turned into linseed oil, a product used as a nutritional supplement and as an ingredient in many wood-finishing products. Flaxseed extract is used to cure disease like peptic ulcer. Flaxseed is known to contain phytochemicals like ferulic acid, linamarin, P-coumaric acid, salicylic acid, sinapic acid, syringic acid, vanillic acid etc. [3] There is high possibility that these phytochemicals play a major role in curing peptic ulcer. However, there is no report identifying the specific phytochemical responsible to cure peptic ulcer.

A group of bacteria belonging to family Helicobacteraceae generally cause Ulcer. Helicobacter pylori, previously known as Campylobacter pylori, is a gram-negative, helically-shaped, microaerophilic bacterium usually found in the stomach. [4] Ulcer is a disease that primarily is a break in the inner lining of the stomach, the first part of the small intestine, or sometimes the lower esophagus. An ulcer in the stomach is called a gastric ulcer, while one in the first part of the intestines is a duodenal ulcer. The most common symptoms of a duodenal ulcer are waking at night with upper abdominal pain and upper abdominal pain that improves with eating [5] [6]. This study focuses on the identification of the phytochemical of *Linum usitatissimum* responsible to cure peptic 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*Linum usitatissimum* contains ferulic acid, linamarin, P-coumaric acid, salicylic acid, sinapic acid, syringic acid, vanillic acid etc. It has already been established that *Linum usitatissimum* plant belonging to Linaceae family has potential to help controlling peptic ulcer. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of pepti ulcer.

Enzyme Found in *Helicobacter pylori*

It has been reported that peptic ulcer 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 alcohol dehydrogenase enzyme (protein database code 3TWO) 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 *Linum usitatissimum* plant were downloaded from the website [7]. The protein database code of the alcohol dehydrogenase enzyme was identified from the website [8]. 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 [9]. Table 1 shows that alcohol dehydrogenase- P-coumaric acid interaction has the highest positive value of -CDOCKER energy (30.013) and minimum value of the difference (1.929) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that P-coumaric acid can effectively deactivate the alcohol dehydrogenase enzyme there by interrupting the biological cycle of *Helicobacter pylori*. Higher positive values for p-coumaric acid indicated that it was the most active ingredient against alcohol dehydrogenase followed by ferulic acid, sinapic acid, vanillic acid, syringic acid, salicyhlic acid and linamarin acid. Thus, the key phytochemical preventing peptic ulcer caused by *Helicobacter pylori* is p-coumaric acid.

CONCLUSION

It was previously known that *Linum usitatissimum* plant has medicinal action against peptic ulcer. Peptic ulcer 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 (ferulic acid, linamarin, P-coumaric acid, salicylic acid, sinapic acid, syringic acid, vanillic acid), which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe. It was found that p-coumaric acid can form strong bond with the enzyme followed by ferulic acid,sinapic acid,vanillic acid, syringic acid, salicyhlic acid and linamarin acid successfully inhibiting the metabolic cycle of the microbe. Thus, this

study could explain that the presence of p-coumaric acid provided the medicinal values to *Linum usitatissimum* against peptic 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	P-Coumaric Acid	30.5503	32.4793	1.929
2	Ferulic Acid	30.013	34.8233	4.8103
3	Sinapic Acid	29.4184	40.121	10.4184
4	Vanillic Acid	26.1716	28.8696	2.698
5	Syringic Acid	24.7169	30.511	5.7941
6	Salicylic Acid	20.1299	22.8876	2.7577
7	Linamarin Acid	10.7462	40.7344	29.9882

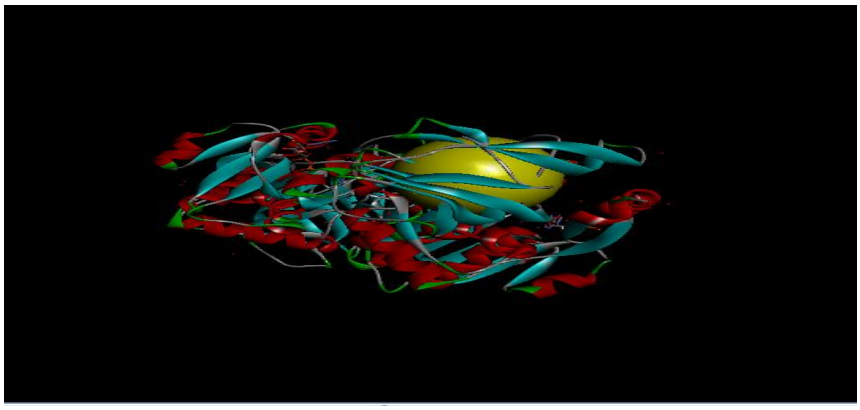

Figure 1. Active Site of Alcohol Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Cymbopogon citratus* against Shikimate Dehydrogenase of *Haemophilus influenzae* Causing Sore Throat

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ABSTRACT

Plants have useful components like phytochemical and biochemical compounds perform different biological functions. The experimental conclusion has proved that *Cymbopogon citratus* plant extract is used for sore throat against its causing agent *Haemophilus influenzae*. The plant extract contains different phytochemicals. 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. The results after study the interaction most of the Phytochemicals deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Haemophilus influenzae*.

Key Words: phytochemical, Biovia, Discovery studio, *Cymbopogon citratus*, *Haemophilus influenzae.*

INTRODUCTION

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants thanks to the traditional belief which says plants are created to provide man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for his or her primary healthcare needs Medicinal plants are the "backbone" of traditional medicine, which suggests quite 3.3 billion people in the less developed countries utilize medicinal plants on a daily basis (1). 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. Phytochemicals generally originated from the plant source are nothing but the bioactive compounds also known as secondary metabolites. Secondary metabolites produced by plants may have little need for them. These are synthesize in almost all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc.(2).

Cymbopogon belongs to family Poaceae. *Cymbopogon citratus* (Lemon grass) may be a widely used herb in tropical countries, especially in Southeast Asia. The volatile oil of the plant is employed in aromatherapy. Lemongrass is equally versatile in the garden. This tropical grass grows in dense clumps which will grow to six ft (1.8 m) tall and about 4 ft (1.2 m) in breadth, with a brief rhizome. (3) The chemical composition of the volatile oil of *Cymbopogon citratus* varies consistent with the geographical origin, the compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered (4). The essential oil (0.2-0.5%, West Indian lemon grass oil) consists of, mainly, citral (5). Citral may be a mixture of two stereoisomeric monterpene aldehydes; the Trans isomer geranial (40-62%) dominates over the cis isomer neral (25-38%). *Cymbopogon* leaves extract is used to cure disease like sore throat.

Cymbopogon mostly contain phytochemicals like Anthraquinones, Apiginin, Caumarins, Citral, Furfurol 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. Sore throat may be a common reason for people consulting general practitioners. Evidence shows that the majority are viral, self limiting, easily self managed, and don't require antibiotics. A group of bacteria belonging to genus *Haemophilus influenza* generally causes sore throat. *Haemophilus influenzae* (formerly called Pfeiffer's bacillus or *Bacillus influenzae*) may be a Gram-negative, coccobacillary, facultatively anaerobic pathogenic bacterium belonging to the Pasteurellaceae family (7). *Haemophilus influenzae* normally exists as a commensal within the human upper tract**,** but can cause disease, either by invasion of the blood stream or by contiguous spread. This study focuses on the identification of the phytochemical of *Cymbopogon* responsible to cure sore throat 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 *Cymbopogon citratus* contains Anthraquinones, Apiginin, Caumarins, Citral, Furfurol etc. It has already been established that *Cymbopogon* plant belonging to Poaceae 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 influenzae.* bacteria. It has been found that shikimate dehydrogenase enzyme (protein database code1P74) is involved in Biosynthesis of antibiotics (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 citratus* 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 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-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 followed by Apiginin, Anthraquinones, coumarins . Thus the results indicated that Furfurol and Apiginin can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Haemophilus influenzae*. Higher positive values for Furfurol indicated that it was the most active ingredient against *Haemophilus influenzae*. On the other hand Citral can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing sore throat caused by *Haemophilus influenza*e are Furfurol.

CONCLUSION

According to previous medical data Sore throat is caused by *Haemophilus influenzae.* Later by theoritical study people came to know that *Cymbopogon citratus* plant has medicinal action against sore throat. Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical

(Anthraquinones, Apiginin, coumarins, Citral, Furfurol), which can have a significant interaction with the vital enzyme (shikimate dehydrogenase) of the microbe. It was found that Furfurol and Apiginin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Citral was found to be not much effective in deactivating the enzyme of the microbe. The rest two Phytochemicals moderately effective to cure sore throat.Thus, this study could explain that the presence of Furfurol provided the medicinal values to *Cymbopogon citratus* against sore throat 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	Anthraquinones	17.091	22.5775	5.4865
2	Apiginin	18.5481	52.6705	34.1224
3	coumarins	15.7693	18.9411	3.1718
4	Citral	-11.2812	27.2834	38.5646
5	Furfurol	19.2704	19.0003	0.2701

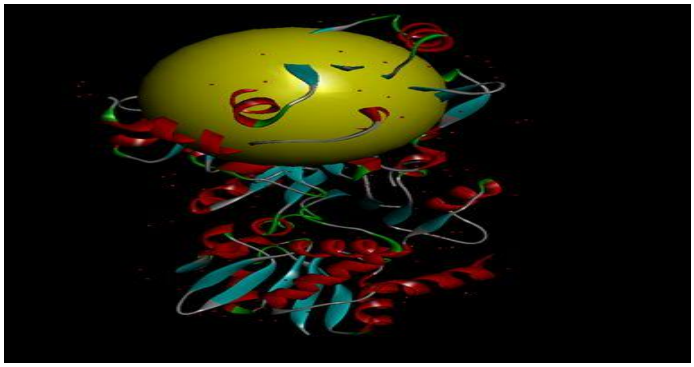

Figure 1. Active Site of Shikimate Dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Marigold against Sterol 14-Alpha Demethylase of *Trichophyton sp* Causing Jock Itch

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ABSTRACT

This analysis aims at evaluating the effects of Marigold extract on Jock itch. Jock itch is caused by *Trichophyton sp*. The phytochemicals of Marigold were interacted with a particular enzyme involved in particular metabolic pathways of *Trichophyton sp.* The enzyme was taken as receptor and phytochemicals were considered as ligands. All the interactions were done in Biovia discovery Studio 2020 and the process is known as molecular Docking. Molecular Docking provides us an opportunity to identify the potential phytochemical or component which can act as powerful tool against the pathogen. Out of all the phytochemicals, Isoquercetin of Marigold inihibts or blocks the mechanism of action of sterol 14-alpha demethylase enzyme of *Trichophyton sp.* There is high possibility that these phytochemicals can potentially inhibit others enzymes involved in various metabolic pathways of *Trichophyton sp.*

Key Words: phytochemical, Biovia Discovery studio 2020, Marigold, metabolic pathways, Jock itch, *Trichophyton sp*

INTRODUCTION

When God created earth he blessed us with a boon called plants. Plants play an important role in sustaining life on earth. They are the soul providers. They provide us a lot of things from Oxygen to food, from timber to firewood and medicines. Role of plants in our life isn't a new concept. We, the humans since the nomadic age have relied on plants for the various purposes. From the very beginning the trees were the soul providers on the earth. Our forefathers relied more on nature than western medicine for their ailments. Use of plants for curing diseases has been mentioned in various Vedas. Ayurveda is well known system of treating diseases with the help of substances taken from nature. In Ayurveda Plants play a major role as most of the constituents or ingredients are taken from plant. From roots to

leaves, from stems to bark, from resins to secondary metabolites every part of the plant can be used in Ayurveda for treatment of diseases. The only demerit the Ayurveda faced was its efficacy. The mode of action was slow. Thus the person suffering from disease did not get fast recovery.

We belong to the 21st century. Life is never slow here. Speed excites us. We love it when our work is done in less span of time. Lifestyle matters most in this era. We consume more junk foods or fast foods which is harmful to our health. With change in lifestyle our body also changes. When we suffer from any kind of disease we depend on chemical drug for fast relief from that disease. The chemical based drugs may provide the fast relief but we don't know about the cost our body is paying for the fast recovery using chemical drugs. The chemical drugs may pretend as a friend from outside but on inside they prove harmful to our systems. In the long run our systems will start failing as a side effect to chemical drugs.

Therefore the people are switching to plant based drugs. Plant based drugs on other hands is a safer alternative to chemical drugs. Plant based drugs are a wonderful combination of nature and science. With the help of science we identify the important component of the plant extract which have potential to inhibit the pathogenic activity of the microbe and try to deal with various component of plant extract. The plant based drugs will have the same efficacy as that of chemical drugs but without any adverse effect.

Marigold belongs to the family Asteraceae. Studies have revealed that Marigold contains Phytochemical like quercetagetin, syringic acid, methyl-3,5-dihydroxy-4- methoxy benzoate, phenolics, Isoquercetin, thienyl, ethyl gallate and Lutein.(1) Lemongrass is known to treat diaper rashes, decrease discoloration, treat wounds,treat bug bites, treat burns, reduce varicose veins,reduce dermatitis and eczema.(2) Jock itch is caused by *Trichophyton sp. Trichophyton sp* is most commonly known to cause dermatophytosis and about 80% cases this pathogen is involved.(3) This study focuses on the identification of the phytochemical from Marigold responsible to cure Jock itch caused by *Trichophyton sp.*

MATERIALS AND METHODS

Software used

All the operations were carried out in Discovery studio module of Biovia 2020 software (Dassault Systemes of France). Biovia 2020 discovery studio is one of the user-friendly software. Its user interface is quite easy to carry out the molecular docking.The software utilizes machine learning techniques to predict the level of molecular interaction between the receptor (enzyme) and Ligand (Phytochemicals).

List of Phytochemicals

Plants produce a number of chemicals which may or may not be directly involved in their metabolism. Phytochemicals are the secondary metabolites produced by plants as a response to flight or fight mechanism against their predators. Phytochemicals are generally bio-active compounds which can affect animal biochemistry and metabolism. Hence they are widely examined to prove their ability towards our health benefits. It becomes important for us to include them in our foods, as potential nutritionally active ingredients. When we consume them they passed on to our systems from plant products. Published works showed that Marigold contains quercetagetin, syringic acid, methyl-3,5-dihydroxy-4- methoxy benzoate, phenolics, quercetin, thienyl, ethyl gallate and Lutein.(4) It has already been established that Marigold plant belonging to Asteraceae family has potential to help controlling Jock itch. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Jock itch.

Enzyme Found in *Trichophyton sp* Jock Itch

From published books and papers we can say that Jock itch is caused due to *Trichophyton sp* infestation. (5) The survival of pathogen inside its host is highly dependent on certain metabolic pathways. These metabolic pathways require certain enzymes as its co-factor to function properly. Brenda enzyme database helped us to identify and list different enzymes found in *Trichophyton sp* causing Jock itch. It has been found that sterol 14-alpha demethylase (protein database code 5TZ1) is involved in steroid biosynthesis metabolism (KEGG). This metabolism proves to be very crucial for the pathogen thus blocking or inhibiting that pathway results in death of the particular microbe.

Molecular Docking

Molecular docking method has been used to identify the phytochemical from the plant extract, which act as a ligand and forms a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia 2020 software was used for identifying molecular interaction and perform molecular docking. First step involves making a list of phytochemicals present in Marigold from various research papers. Second steps involves download of the sdf files for the phytochemicals found in the Marigold plant from various website like PubChem, Mol Instincts etc. The protein database code of sterol 14-alpha demethylase enzyme was identified from the RCSB-PDB 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 sterol 14-alpha demethylase 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 (6).

Table 1 shows that sterol 14-alpha demethylase- Quercetin interaction has the highest positive value of -CDOCKER energy (24.7971) and minimum value of the difference (3.4569) between - C DOCKER interaction energy and - C DOCKER energy followed by Isoquercetin. Thus the results indicated that Quercetin and Isoquercetin can effectively deactivate the enzyme thereby interrupting the biological cycle of *Trichophyton sp*. Higher positive values for Quercetin indicated that it was the most active ingredient against *Trichophyton sp*. On the other hand, alpha- pienecan deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing Jock itch caused by *Trichophyton sp* Jock itche sp. are Quercetin and isoquercetin.

CONCLUSION

Chemical based drug are efficient but prove to be harmful in long run. Plant based drugs have are safer alternate as they don’t harm us. One of the important plants that can be used for plant based drugs is Marigold .Marigold is a well-known for its aromatic tea and volatile oils. Marigold pot extracts can be used as diaphoretic, antispasmodic, stimulant, anti-pyretic agents and antiseptic. (7) It was previously known that Marigold plant has medicinal action against Jock itch. Jock itch is caused by *Trichophyton sp*. This study was carried out to provide the theoretical basis of

this observation. Using Discovery studio module of Biovia 2020 software, molecular docking operation was performed to identify the phytochemicals (Quercetin and Isoquercetin) which can have a significant interaction with the vital enzyme (sterol 14-alpha demethylase) of the microbe. It was found that Quercetin and Isoquercetin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha- piene, Alpha-Thujene and Erythrodiol were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Quercetin and Isoquercetin provided the medicinal values to Marigold against Jock itch caused by *Trichophyton sp*. But we can also conclude that other phytochemicals may or may not inhibit other enzymes present in other biological cycles of *Trichophyton sp.*

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Table1: Results of Cdocking Of Phytochemicals With Sterol 14-Alpha Demethylase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Quercetin	24.7971	28.254	3.4569
2	Isoquercetin	8.52127	43.7017	35.18043
3	Alpha-piene	-16.4734	8.50165	24.97505
4	Alpha- thujene	-42.2304	11.2567	53.4871
5	Erythrodiol	-60.7807	36.1174	96.8981

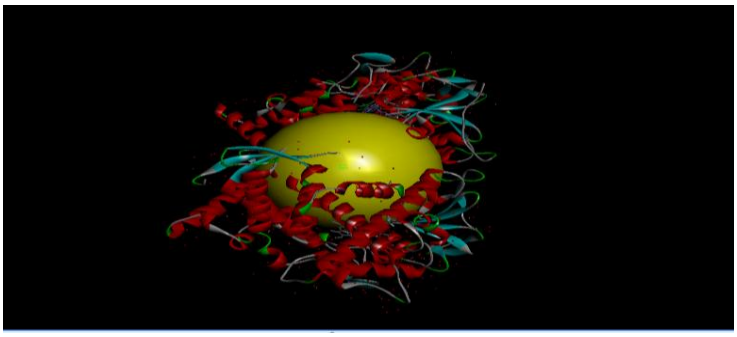

Figure 1: Active Site of Sterol 14-Alpha Demethylase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Glycyrrhiza glabra* (Mulethi) against Alcohol Dehydrogenase of *Helicobacter pylori* Causing Ulcer

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ABSTRACT

Phytochemicals are substances, produced by plants and these substances have biological activity. Phytochemicals are non-nutritive plant chemicals that contain protective, disease preventing compounds. It has been reported that *Mulethi* plant extract is used to cure ulcer. The plant extract contains different phytochemicals. Extraction of aqueous in *Mulethi* plant have high amount of phytochemicals and high antioxidant activity. 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 3D structure of the targets were retrieved from research collaboratory of Structural Bioinformatics Protein data bank (RCSB PDB). The PDB ID and sdf files were used in the discovery studio module of BIOVIA software and interaction between then were analysed. 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 Rosmarinic acid, Pectin, Catechin, 3 Bromo-2-pentanol can effectively deactivate the enzyme thereby interrupting the life cycle of *Helicobacter pylori*.

Key Words: Phytochemical, BIOVIA, Discovery studio, *Mulethi, Helicobacter pylori.*

INTRODUCTION

Life is much harder in olden days, but it would have been more peaceful in Olden days. Today in Modern times life is different from old on all sides; Comfortable, Physical conditions, readymade and unhealthy. Changing work condition, less physicalactivity, sedentary jobs, comfortable but stressful life and bad eating habits has exposed us tosome dangerous health hazards like blood pressure, diabetes, obesity etc. The change into modern life is positive

and should be continued in control. Cautions and awareness prevent these lifestyle related diseases from increasing. Nature has been a source of medicinal agents. An impressive number of modern drugs have been derived from natural source (Heinrichs Metal., 2010). Modern drugs have been isolated from natural sources, which is based on their use in traditional medicine. 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. (Devi P.R., 2014). The bioactive compounds derived from natural resources, its phytochemicals analysis, characterization and pharmacological investigations. These drug compounds can be useful for human resources. Plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

A medicinal plant is used with the intention of maintaining health to be administered for a specific condition whether in modern medicine or traditional medicine. 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., vincristine 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. The quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required in research. Newly emerging scientific techniques and approaches have been used in research, for the investigation of constituents and determination of biological activity of medicinal plants.

Many plants produce special substances in their roots, leaves, flowers or seeds that help them to survive. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Drugs that includes antibiotics, antifungals, antiprotozoal and antivirals called antimicrobial. An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial activities of solvent extracts of *Mulethi* were determined by disc diffusion method on Muller Hinton Agar medium. *Mulethi* belongs to family Fabaceae. *Mulethi* leaves extract is used to cure disease like Ulcer. *Mulethi* is known to contain phytochemicals like Phloretin-3-5-Di-C-glucoside, Cornstarch,3-Bromo-2-Pentanol, Rosmarinic acid, (+) Catechin, 1-Hexanol, liquiritigenin, Linalool-oxide-A, tanicacid, cortison, Geraniol, Sucrose, cholesterol, Isocoumarin, Pyrazine, Harmalol, glycerrehetinic acid, glycerrehizin, Terpinen-4-ol, alpha-Terpineol, Pectin etc **(**reference).

There is high possibility that these phytochemicals play a major role in curing ulcer. However, there is no report identifying the specific phytochemical responsible to cure ulcer. A group of Helicobacter pylori belonging to family Helicobacteraceae generally cause ulcer. They are spiral shaped Gram-negative bacteria. Ulcer is a common bacterial disease that affects the stomach. For mouth ulcers cause due to ailment. *Mulethi* helps in cleaning stomach and removes toxins that could be responsible for ulcer. *Mulethi* has anti-inflammatory effects and anti-ulcer properties. It decreases the inflammation of stomach linings. It has protective effects against stomach ulcer. It reduces the chances of gastric ulceration induced by aspirin and other NSAIDs. In duodenal ulcer, deglycyrrhizinized licorice

(Yashtimadhu) has ulcer healing properties. It corrects the gastric mucosa and reduces ulcerations. This study focuses on the identification of the phytochemical of Mulethi responsible to cure Ulcer caused by *Helicobacter pylori.*

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 *Mulethi* contains Phloretin-3-5-Di-C-glucoside, Cornstarch,3-Bromo-2-Pentanol, Rosmarinicacid, (+) Catechin,1-Hexanol, liquiritigenin, Linalool-oxide-A, tanicacid, cortison, Geraniol, Sucrose, cholesterol, Isocoumarin, Pyrazine, Harmalol, glycerrehetinic acid, glycerrehizin, Terpinen-4-ol, alpha-Terpineol, Pectin etc. It has already been established that *Mulethi* 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 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*. It has been found that Alcohol dehydrogenase 3TWO is involved in Methionine 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 Mulethi plant were downloaded from the website. The protein database code of Alcohol 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 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 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 32.2325-7.26983 interaction has the highest positive value of -CDOCKER energy 24.96265 and minimum value of the difference 2.7732 between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that 24.96265 can effectively deactivate the enzyme thereby interrupting the biological cycle of *Helicobacter pylori.* Higher positive values for indicated that it was the most active ingredient against Alcohol dehydrogenase of Helicobacter pylori. On the other hand, Alcohol dehydrogenase can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Liquiritin, tanic acid cannot interact with *Helicobacter pylori.* Alpha terpeniol, Berberine interact with enzyme very loosely. Thus, the key phytochemicals preventing ulcer caused by *Helicobacter pylori* are Catechin and Rosmarinic acid, pectin**.**

CONCLUSION

It was previously known that *Mulethi* plant has medicinal action against ulcer. Ulcer 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 Phloretin-3-5-Di-C-glucoside, Cornstarch, 3-Bromo-2-Pentanol, Rosmarinicacid, (+)Catechin, 1-Hexanol, liquiritigenin, Linalool-oxide-A, tanicacid, cortison, Geraniol, Sucrose, cholesterol, Isocoumarin, Pyrazine, Harmalol, glycerrehetinic acid, glycerrehizin, Terpinen-4-ol, alpha-Terpineol, Pectin**.** which can have a significant interaction with the vital enzyme Alcohol dehydrogenase of *Helicobacter pylori.* It was found that pectin, Catechin, Rosmarinic acid and 3-Bromo-2-Pentanol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Berberine, alpha-Terpineol were found to be not much effective in deactivating the enzyme of the microbe. Tanic acid, Liquiritin, cornstarch cannot deactivate the enzyme. Thus, this study could explain that the presence of Pectin, Catechin and Rosmarinic acid provided the medicinal values to Mulethi against ulcer caused by *Helicobacter pylori.* The vast range of biological effects like anti-inflammatory, anti-allergic, anti-oxidant, anti-viral of the phytochemicals present in extract have been of immense importance in phototherapeutic *Mulethi* or licorice grows perfectly in well-drained soil which is situated in the deep valleys experiencing complete sunlight

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

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTION ENERGY AND -CDOCKER ENERGY
1	(+)-Catechin	32.5147	51.0821	18.5674
2	3-Bromo-2-Pentanol	18.2271	20.1908	1.9637
3	Rosmarinic acid	45.6245	48.3977	2.7732
4	Liquiritin	Failed	Failed	
5	Pectin	7.26983	32.2325	24.96265
6	alpha-Terpineol	-2.00985	26.3512	28.36105
7	tanicacid	Failed	Failed	
8	Cornstarch-3D-Structure	Failed	Failed	
9	Berberine	-50.09285	30.7675	80.8603

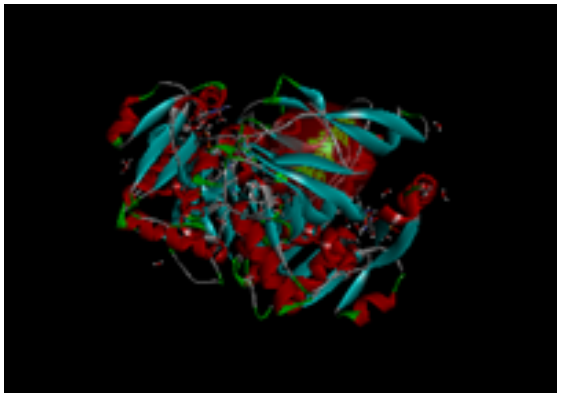

Figure 1: Active Site of Alcohol Dehydrogenase Enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Myristica fragrans* against Superoxide Dismutase of *Porphyromonas gingivalis* Causing Periodontitis

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Myristica fragrans* plant extract is used to cure Periodontitis. The plant extract contains different phytochemicals. Periodontitis is caused by *Porphyromonas gingivalis.* One of the key enzymes involved in its biochemical pathway is Superoxide dismutase. 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 Docosane and Elemicin can effectively deactivate the Superoxide dismutase enzyme thereby interrupting the life cycle of *Porphyromonas gingivalis* .

keywords: phytochemical, Biovia, Discovery studio, *Myristica fragrans,Porphyromonas gingivalis.*

INTRODUCTION

In earlier time before the production of chemical medicines, man was dependent on the medicinal plants. Due to the medicinal properties of plants some people value these plants. The ancient belief which says plants are there for humans to supply them food, medical treatment, and other needs. It is thought that about 80% of the 5.2 billion people of the planet sleep in the less developed countries and therefore the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for his or her primary healthcare needs (1). Phytochemicals are chemical compound in plants which are found in various parts like leaves, vegetables

and roots that have defense mechanism against various diseases. Phytochemicals are not only primary but also secondary compounds.The primary constituents are Chlorophyll, proteins and common sugars and secondary compounds have terpenoid, alkaloids and phenolic compounds. (2).

Terpenoids have various important role in medicinal activities activities i.e., anticancer, anti-inflammatory, anti-malarial, anti-viral and anti-bacterial activities, inhibition of cholesterol synthesis (3). Terpenoids are used for attracting useful mites and consume the herbivorous insects (4). Alkaloids are naturally occurring oganic compounds that mostly contains nitrogen atoms, are found in medicinal plants. (5) *Myristica* belongs to family Myristicaceae, mostly found in Malaysia, West Indies and Kerala state of India. The native of nutmeg is Moluccas Island and it is cultivated throughout, Karnataka, parts of TamilNadu, Goa, Assam, Kerala and Andaman and Nicobar Islands in India. The tree grows upto 25 feet high & bloom when it is nine years old. It is used for its flavoring and medicinal properties (6). *Myristica* leaves extract is used to cure disease like Periodontitis (7). *Myristica* is known to contain phytochemicals like α-Cubebene, α-pinene, α-phellandrene, Docosane, Elemicin etc.(8).

There is high possibility that these phytochemicals play a major role in curing Periodontitis. However, there is no report identifying the specific phytochemical responsible to cure Periodontitis. Periodontitis is caused by *Porphyromonas gingivalis* (9). *Porphyromonas gingivalis* is a Gram-negative bacteria that causes periodontitis, an inflammatory disease that destroys the tissues supporting the tooth which eventually may lead to tooth loss (10). This study focuses on the identification of the phytochemical of *Myristica* responsible to cure Periodontitis caused by *(Porphyromonas gingivali).*

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 *Myristica fragrans* contains α-Cubebene, α-pinene, α-phellandrene, Docosane, Elemicin etc. It has already been established that *Myristica indica* plant belonging to Myristicaceae family has potential to help controlling Periodontitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Periodontitis.

Enzyme Found in *Porphyromonas gingivalis*

It has been reported that Periodontitis 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 superoxide dismutase enzyme (protein database code1QNN) is involved in various metabolic pathways (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 *Myristica fragrans* plant were downloaded from the website. The protein database code of the Superoxide dismutase 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 Superoxide dismutase 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 (11). Table 1 shows that Superoxide dismutase-Docosane interaction Superoxide dismutase has the highest positive value of -CDOCKER energy (40.2479) and minimum value of the difference (1.6448) between - C DOCKER interaction energy and - C DOCKER energy followed by Elemicin. Thus the results indicated that Docosane and Elemicin can effectively deactivate the Superoxide dismutase enzyme thereby interrupting the biological cycle of *Porphyromonas gingivalis.* Higher positive values for Docosane indicated that it was the most active ingredient against *Porphyromonas gingivalis*. On the other hand α-Cubebene, α-pinene,α-phellandrene can deactivate the enzyme to a small extent (negative -CDocker energy but positive-CDocker interaction energy). Thus, the key Phytochemicals preventing Periodontitis caused by *Porphyromonas gingivalis* are Docosane and Elemicin.

CONCLUSION

It was previously known that *Myristica fragrans* plant has medicinal action against Periodontitis. Periodontitis 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 (α-Cubebene, α-pinene, α-phellandrene, Docosane, Elemicin), which can have a significant interaction with the vital enzyme Superoxide dismutase of the microbe. It was found that Docosane and Elemicin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. α-Cubebene, α-pinene, α-phellandrene were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Docosane and Elemicin provided the medicinal values to *Myristica fragrans* against Periodontitis caused by *Porphyromonas gingivalis.*

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Table 1. Results of Cdocking of Phytochemicals with Superoxide Dismutase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	α-Cubebene	-29.6193	28.0955	57.7148
2	α-pinene	-9.004	16.0869	25.0909
3	α-phellandrene	-12.3214	17.404	29.7254
4	Docosane	40.2479	38.6031	1.6448
5	Elemicin	6.44075	28.9743	22.53355

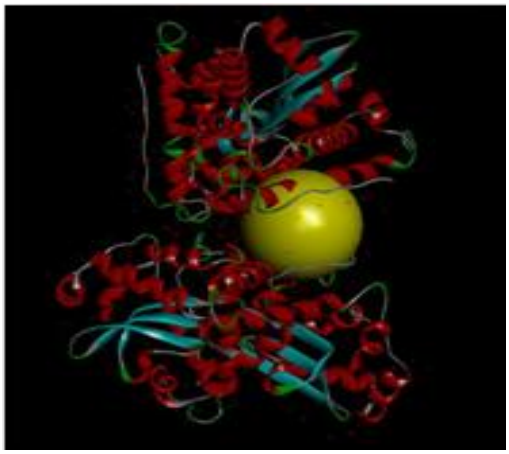

Figure1. Active Site of Superoxide Dismutase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Tomato Flakes against Peroxiredoxin of *Staphylococcus aureus* Causing Eczema

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that Tomato Flakes extract is used to cure Eczema. The plant extract contains different phytochemicals. Eczema is caused by *Staphylococcus aureus.* One of the key enzymes involved in its biochemical pathway isPeroxiredoxin. 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 Peroxiredoxin enzyme thereby interrupting the life cycle of *Staphylococcus.*

Key Words: phytochemical, Biovia, Discovery studio, Tomato flakes, *Staphylococcus 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.

Tomato flakes belongs to family Solanaceae (Wikipedia). Tomato flakes extract is used to cure disease like diarrhoea, dysentery, eczema. Tomato flakes is known to contain phytochemicals like kaempferol, naringenin, lycopene, quercetin, phytoene etc.(Tomato phytochemicals and prostates cancer risk the journal of nutrition oxford academic), (https://www.thehealthsite.com)There is high possibility that these phytochemicals play a major role in curing Eczema. However, there is no report identifying the specific phytochemical responsible to cure Eczema. A group of bacteria belonging to genus *Staphylococcus* generally cause Eczema. It is a round shaped Gram-positive bacterium. They are found in the human upper respiratory tract and on the skin. This study focuses on the identification of the phytochemical of Tomato flakes responsible to cure Eczema caused by *Staphylococcus.*

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 Tomato flakes contains kaempferol, naringenin, lycopene, quercetin, phytoene, etc. It has already been established that Tomato flakes belonging to Solanaceae 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* 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*. It has been found that Peroxiredoxin (protein database code 3P7X) 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 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 Tomato flakes were downloaded from the website. The protein database code of the Peroxiredoxin 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.

RESULTS AND DISCUSSION

Fig. 1 shows the active site of Peroxiredoxin 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 Peroxiredoxin-Quercetin interaction has the highest positive value of -CDOCKER energy (32.1785) and minimum value of the difference (4.1695) between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that Quercetin can effectively deactivate the enzyme Peroxiredoxin thereby interrupting the biological cycle of *Streptococcus aureus*. Higher positive values for indicated that it was the most active ingredient against *Streptococcus aureus*. On the other hand, Kaempferol & Naringenin can deactivate the enzyme to a small extent. Lycopene& Phytoene cannot interact with Peroxiredoxin. Thus, the key phytochemicals preventing Eczema caused by *Staphylococcus aureus* are Quercetin and Kaempferol.

CONCLUSION

It was previously known that Tomato flakes 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 phytochemical

(Kaempferol, Naringenin, Lycopene, Quercetin, Phytoene), which can have a significant interaction with the vital enzyme Peroxiredoxin of the microbe. It was found that Quercetin and Kaempferol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Naringenin was found to be not much effective in deactivating the enzyme of the microbe. Lycopene & Phytoene cannot deactivate the enzyme. Thus, this study could explain that the presence of Quercetin and Kaempferol provided the medicinal values to Tomato flakes against Eczema caused by *Staphylococcus aureus*.

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

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Kaempferol	26.982	33.6027	6.6207
2	Naringenin	26.6464	32.0761	5.4297
3	Lycopene	NA	NA	NA
4	Quercetin	32.1785	36.348	4.1695
5	Phytoene	NA	NA	NA

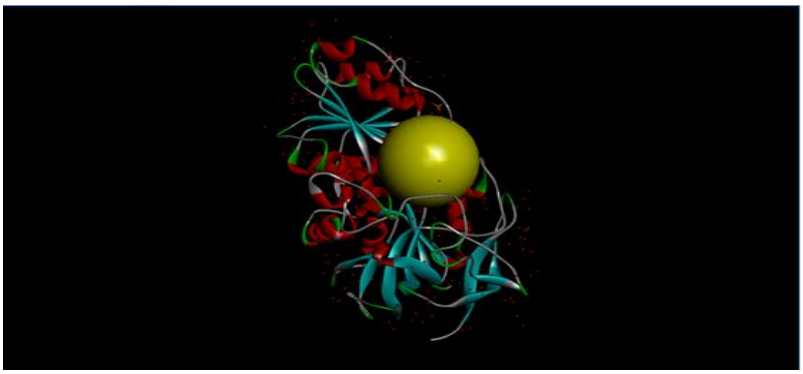

Figure 1. Active Site of Peroxiredoxin Enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Polianthes tuberosa* against *Staphylococcus aureus* Causing Skin Disease

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *polianthes tuberosa* plant extract is used to cure skin disease. The plant extract contains different phytochemicals. Skin disease is caused by *Staphylococcus aureus.* Two key enzymes involved in its biochemical pathway is L-lactate dehydrogenase and IMP dehydrogenase. The molecular docking of the phytochemicals with the enzymes 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 on protein database code 3D4P, phytochemicals Kameferol 3-o and Alpha-d glucoside can effectively deactivate the L-lactate dehydrogenase enzyme and on protein database code 6I0O, kameferol 3-o can effectively deactivate the IMP dehydrogenase enzyme thereby interrupting the life cycle of staphylococcus.

Key Words: Phytochemical, Biovia, Discovery studio, *Polianthes tuberosa, Staphylococcus aureus.*

INTRODUCTION

Olden life style was natural, slow but healthy. Modern lifestyle pattern is readymade, less physical activity, sedentary job, work condition changing, comfortable but stressful life and bad eating habits which have negative effect on health physically, psychologically, and socially. These life style increases the risk of obesity, consequently, leading to diabetes, heart disease, cancer and high blood pressure. This is particularly correct about the new evolution of the new development of the medicines and vaccines that save people from various diseases. 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 plants are useful for curing of human diseases because of the presence of

phytochemical constituents. Phytochemicals are chemical compounds which occur naturally in the medicinal plants, bark, leaves, flowers, roots, fruits, vegitables and seeds that have defence mechanisms and can be used for therapeutic purposes. (Khan et al, 2013). The present study was carried out to evaluate the anti-cancer, anti-oxidant, anti-inflammatory and anti-microbial properties of various medicinal plants frequently used in the Indian traditional medication. (Shaikh et al.2014). The emphasis on the use of medicinal plants had hitherto been placed on the treatment rather than prevention of diseases, medicinal plant play a key role on human health care (Sofowora et al.2013). 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. (Fatima et al, 2012). They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (Singh, 2015). 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. Approximately one half of all licensed drugs were registered worldwide in the 25yr. period to 2007 were natural products are their synthetic derivatives. (Kennedy et al. 2011). 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 have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Bioassay are adaptable for screening and testing plant extracts. (Karakas et al. 2012).

Polianthes tuberosa is commonly known as Rajanigandha in India due to its night blooming fragrant flower. The name polianthes derived from 'polis' which means white and 'anthers' which means flower. Tuberose flower is a rich source of essential oils with pleasant sweet aroma which has a wide range of application in perfumery and cosmetics industry (Rammamurthy et al., 2010) . There are about 15 species under genus Polianthes, of which 12 species have been reported from Mexico and Central America. Of these 9 species have white flower; one is white tinged with red and two are red. *Polianthes tuberose* belongs to family Asparagaceae. Leaves extract is used to cure disease like skin diseases. The leaves of *Polianthes tuberosa* were show to contains phytochemicals like Alpha-D-glucoside, Kameferol 3-o. There is high possibility that these phytochemicals play a major role in curing skin disease. However, there is no report identifying the specific phytochemical responsible to cure skin disease.

A group of bacteria belonging to genus *Staphylococcus* generally cause skin disease. *Staphylococcus aureus* are gram positive, round-shaped bacterium that are member of the Firmicutes and usual member of microbiota of the body and Staphylococcus belongs to family *Streptoccaceae* (Creech et al. 2015). *Staphylococcus aureus* frequently found in the upper respiratory tract and on the skin of human body, and its passage is always pathogenic. *Staphylococcus* bacteria can grow on the skin without the need for oxygen. Most of the normal healthy population were affected by *Staphylococcus aureus* as its pathogen is human hosts. (Kobayashi et al. 2015). This bacteria also causes various diseases like skin diseases, Pharyngitis, *Pneumonia* etc. This study focuses on the identification of the phytochemical of *Polianthes tuberosa* responsible to cure skin disease 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 *Polianthes tuberosa* contains alpha-D-glucoside, kameferol 3-o. It has already been established that *Polianthes tuberosa* plant belonging to Asparagaceae family 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 *Staphylococcus aureus*

It has been reported that skin disease can cause as a result of *Staphylococcus aureus* 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* bacteria. It has been found that L-lactate dehydrogenase enzyme (protein database code 3D4P) is involved in L-lactaldehyde degradation, lactate fermentation (KEGG) ,IMP dehydrogenase enzyme (protein database code 6I0O) involved in purine metabolism(www.brendaenzyme.org), Shikimate dehydrogenase enzyme (protein database code 1NYT) involved in Chorismate 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 *Polianthes tuberosa* plant were downloaded from the website (www.sphinxsai.com). The protein database code of the L-lactate dehydrogenase enzyme, IMP dehydrogenase enzyme and 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 L-lactate dehydrogenase enzyme, Fig. 2 shows the active site of the IMP dehydrogenase enzyme, Fig. 3 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 (Brinda et al. 2019). Table 1, shows that on protein database code 3D4P, L-lactate dehydrogenase-kameferol 3-o interaction has the highest positive value of -CDOCKER energy (16.8507) and minimum value of the difference (44.7825) between - C DOCKER interaction energy and - C DOCKER energy followed by Alpha-D glucoside. Thus the results indicated

that Kameferol 3-o and Alpha-D glucoside can effectively deactivate the L-lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Staphylococcus aureus*. Higher positive values for Kameferol 3-o indicated that it was the most active ingredient against *Staphylococcus aureus.* Table 2, shows that on protein Database code 6I0O, IMP dehydrogenase-Kameferol 3-o interaction have less positive value of -CDOCKER energy (6.23114) than L-lactate dehydrogenase-kameferol 3-o interaction and minimum value of the difference (42.71876) between -C DOCKER interaction energy and -C DOCKER energy. Here the results indicated that Kameferol 3-o not much effectively deactivate the IMP dehydrogenase enzyme as compare to the L-lactate dehydrogenase enzyme, on the other hand, Alpha-D glucoside can deactivate the IMP dehydrogenase enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy).

Table 3, shows that on protein database code 1NYT, Shikimate dehydrogenase - Alpha-d glucoside interaction has negative value of -CDOCKER energy (-13.5031) and minimum value of difference (37.0219) between -C Docker interaction energy and -C Docker energy. Thus Alpha-d glucoside can also deactivate the shikimate dehydrogenase enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Kameferol 3-o cannot interact with the Shikimate dehydrogenase enzyme. Thus, from the above 3 tables, on pdb code 3D4P (Table 1), Kameferol 3-o and Alpha-d glucoside can effectively deactivate the L-lactate dehydrogenase due to the highest positive value of -C Docker energy. The key phytochemicals preventing skin disese caused by *Staphylococcus aureus.* are Kameferol 3-o and Alpha-d glucoside.

CONCLUSION

It was previously known that *Polianthes tuberosa* plant has medicinal action against skin disease. Skin disease 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 (Alpha-D-glucoside, Kameferol 3-o). On protein database code 3D4P, these phytochemicals can have a significant interaction with the vital enzyme (L-lactate dehydrogenase) of the microbe. It was found that Kameferol 3-o and alpha -d glucoside can form a strong bond with the enzyme (L-lactate dehydrogenase) successfully inhibiting the metabolic cycle of the microbe. On protein database code 6I0O, Kameferol 3-o was effective where as Alpha-d glucoside was not much effective in deactivating IMP dehydrogenase enzyme of the microbe. On protein database code 1NYT, Alpha-d glucoside was not much effective to deactivate the Shikimate dehydrogenase and kameferol 3-o cannot deactivate the Shikimate dehydrogenase enzyme. Thus this study could explain that on protein database code 3D4P, the presence of Kameferol 3-o and Alpha-d glucoside which interaction with L-lacate dehydrogenase enzyme and on pdb code 6I0O , the presence of kameferol 3-o interaction with IMP dehydrogenase provide the medicinal values to Polianthes tuberosa against skin disease caused by Staphylococcus aureus.

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Table 1. Results of CDocking of Phytochemicals With L-lactate Dehydrogenase (Receptor) Protein database-3D4P

SL NO	LIGAND	-C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTION ENERGY AND -C DOCKER ENERGY
1	Alpha-D glucoside	11.1616	34.5928	23.4312
2	Kameferol 3-o	16.8507	61.6332	44.7825

Table 2. Results of CDocking of Phytochemicals with IMP Dehydrogenase (Receptor) Protein Database-6I0O

SL NO	LIGAND	-C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTION ENERGY AND -C DOCKER ENERGY
1	Kameferol 3-o	6.23114	48.9499	42.71876
2	Alpha-D glucoside	-1.95793	20.7702	22.72813

Table 3. Results of CDocking of Phytochemicals With Shikimate Dehydrogenase Protein Database-1NYT

SL NO	LIGAND	-C DOCKER ENERGY	-C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTION ENERGY AND -C DOCKER ENERGY
1	Alpha-D glucoside	-13.5031	23.5188	37.0219
2	Kameferol 3 o	Failed	Failed	NA

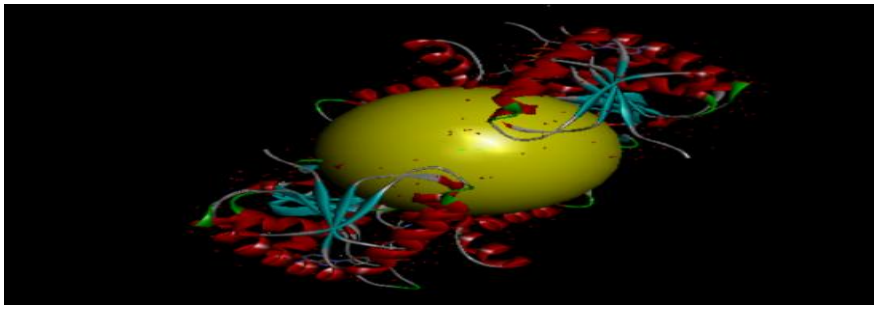

Figure 1. Active site of L-lactate dehydrogenase enzyme

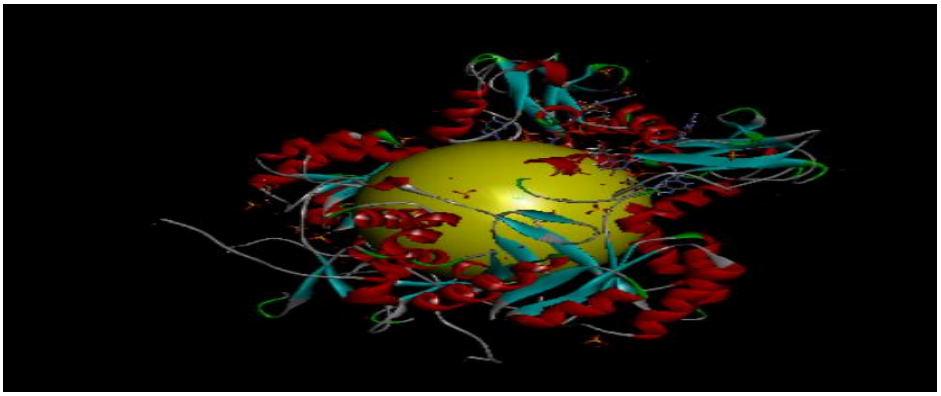

Figure 2. Active site of IMP dehydrogenase

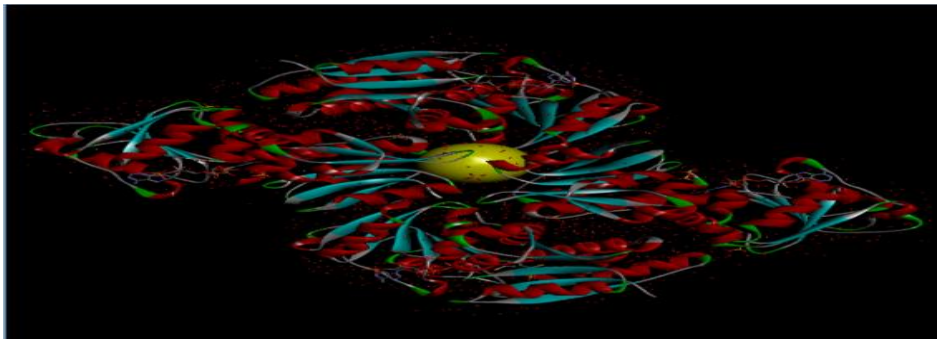

Figure 3. Active site of Shikimate dehydrogenase enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Ocimum tenuiflorum* against Shikimate Dehydrogenase of *Haemophilus influenzae* Causing Sore Throat

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ABSTRACT

One of the most opportunistic pathogens (*Haemophilus influenzae*); they usually live in their host without causing disease, but cause problems only when other factors create an opportunity. They infect the host by using poteins, trimeric autotransporter adhesions, which are found in outer membrane of gram negative bacteria.some stains of *H.influenzae* causes respiratory diseases like sore throat. One of the key enzymes involved in its biochemical pathway is shikimate dehydrogenase. It has been reported that *Ocimum tenuiflorum* plant extract is used to cure sore throat via experiment. The plant extract contains different phytochemicals. The molecular docking of the phytochemicals with the respective 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 maximum number of Phytochemicals present in this plant can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the life cycle of *Haemophilus influenzae*.

Key Words: phytochemical, Biovia, Discovery studio, *Ocimum tenuiflorum*, *Haemophilus influenzae.*

INTRODUCTION

The history tells us that plant resources are impotant part of Human society.After fulfilling the primary desire, these resources help us as remedy for curing various diseases. (1)(2) Modern drugs have been derived from plant source, those plants are known as medicinal plants (3). Those drugs prescribed to patients are rigorously tested (4). Plants based medicinal constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, and seeds

(5). The phytoextracts of various medicinal plants have shown numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes action etc. (6). Medicinal plants play a key role in human health care. Third-fourth of the world population are dependent on theherbal medicine because of their less side effects, efficacy of medicinal plants and their cost effectiveness. (7).

Presently the medicinal plants have the most industrial uses. These range from traditional medicines, herbal teas, and health foods such as nutriceuticals to galenicals, phytopharmaceuticals and industrially produced pharmaceuticals. In pharmaceutical fields the herb foxglove is the source for digitalis and therefore the herb salicin is that 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*. (8)(9). Research needs within the field of medicinal plants are huge, but are balanced by the potential health benefits and therefore the enormous size of the market. Research into the standard, safety, biological activity, and clinical efficacy of the various 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 (10). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Ocimum belongs to family Lamiaceae. *Ocimum* leaves extract is used to cure disease like sore throat. *Ocimum* mostly contain Phytochemicals like Apigenin, Carnosic acid, Eugenol, Luteoline, Rosmarinic acid 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 influenzae* generally causes sore throat. *Haemophilus influenzae* (formerly called Pfeiffer's bacillus or *Bacillus influenzae*) is a Gram-negative, coccobacillary, facultatively anaerobic pathogenic bacterium belonging to the *Pasteurellaceae* family (11). This study focuses on the identification of the phytochemical of *Ocimum responsible* to cure sore throat 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 *Ocimum tenuiflorum* contains Apigenin, Carnosic acid, Eugenol, Luteoline, Rosmarinic acid etc. It has already been established that *Ocimum* plant belonging to Lamiaceae 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 influenza*e

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 influenzae.* bacteria. It has been found that shikimate dehydrogenase enzyme (protein database code1P74) is involved in Biosynthesis of antibiotics (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 *Ocimum tenuiflorum* plant were downloaded from the website (http://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 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- Rosmarinic acid interaction has the highest positive value of -CDOCKER energy(44.6291) and minimum value of the difference (0.9662) between - C DOCKER interaction energy and - C DOCKER energy followed by Luteoline, Apigenin, Carnosic acid, Eugenol. Thus the results indicated that all the Phytochemicals taken in this operation are positively effective for curing diseases by inhibiting that specific enzyme of causative microbe.. Higher positive values for Rosmarinic acid indicated that it was the most active ingredient against *Haemophilus influenzae* although all Phytochemicals are capable and active against *H.influenzae*. Thus, the key phytochemicals preventing sore throat caused by *Haemophilus influenzae* are Apigenin,Carnosic acid, Eugenol, Luteoline, Rosmarinic acid.

CONCLUSION

Sore throat is caused by *Haemophilus influenzae* the study about *Ocimum citratus* plant having medicinal action was carried out previously. Thus, *Ocimum citratus* is used against sore thoat Using Discovery studio module of Biovia software, molecular docking operation was performed to identify the phytochemical (Apigenin,Carnosic acid, Eugenol, Luteoline, Rosmarinic acid), which can have a significant interaction with the vital enzyme (shikimate

dehydrogenase) of the microbe. It was found that all of the above five Phytochemicals are able to form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Thus, this study could explain that the presence of Apigenin,Carnosic acid, Eugenol, Luteoline, Rosmarinic acid provided the medicinal values to *Ocimum tenuiflorum* against sore throat 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	Apigenin	27.4545	32.8235	5.369
2	Carnosic acid	15.2331	36.1289	20.8958
3	Eugenol	11.9311	21.4098	9.4787
4	Luteoline	30.8655	34.1252	3.2597
5	Rosmarinic acid	44.6291	43.6629	0.9662

Figure 1. Active Site of Shikimate Dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Azadirachta indica* against Cinnamyl-Alcohol Dehydrogenase of *Helicobacter pylori* Causing Ulcer

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ABSTRACT

The bioactive compounds, obtained from the plants have vital role in the traditional medicine are named as Phytochemicals. From the previous study it is reported that *Azadirachta indica* plant extract is helpful for ulcer.The Phytochemicals present in Neem plants show more antimiocrobial activity against pathogen than other common plant of India. Ulcer is a gastroeso phageal reflux disease caused by *Helicobacter pylori.* One of the important enzymes involved in its metabolism is cinnamyl-alcohol dehydrogenase. Using Biovia Discovery Studio, the molecular docking of the phytochemicals with the related 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 Glutamic Acid, L-ascorbic acid& Oleic acid can effectively deactivate the cinnamyl-alcohol dehydrogenase enzyme by distrupting the life cycle of *Helicobacter pylori*.

Key Words: phytochemical, Biovia, Discovery studio, *Azadirachta indica, Helicobacter pylori.*

INTRODUCTION

In earlier times, life was undeveloped but healthy. In this machine age, 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 makes us sick and 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 is a source of all the valuable things, which will fulfil our needs in various way. It contains medicinal plants as medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural source (heinrich Metal., 2010). These medicinal agents act as the back bone of traditional medicine (1). The medicinal properties of the plants do not lie in whole body of plant, it not only lies in

some chemical substances that regulates metabolism on the human body, and these substances are called phytochemicals, which can be used for therapeutic purpose. Medicinal constituents can be derived from plant parts 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) More than half of the world population relies mainly on plants and plant products for health care. About one-third of the entire plant species, at one time or other, were used for medicinal purposes. In world market, the value of plant derived drugs may account for about Rs.2,00,000 crores. In present days Indian contribution is less than Rs.2000 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1994-'95 from Rs.130 crores in 1991-'92. The annual production of medicinal and aromatic plant's raw material is worth about Rs.200 crores. This is likely to touch US $1150 by the year 2000 and US $5 trillion by 2050.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 Atropabelladonna 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.

Azadirachta is the well known species of family Meliaceae, It commonly known as neem, has attracted by people for its medicinal quality in recent years. Formerly Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine (2). *Azadirachta* leaves extract is used to cure disease like ulcer (reference). *Azadirachta* is known to contain phytochemicals like Glutamic Acid, L-ascorbic acid, β-sitosterol, Oleic acid, Stigmasterol (2). There is high possibility that these phytochemicals play a major role in curing ulcer. However, there is no report identifying the specific phytochemical responsible to cure ulcer.

A group of bacteria belonging to genus *Helicobacter pylori* generally cause ulcer. They are helical or spiral shaped Gram negative bacteria. *Helicobacter pylori (H. pylori)* is a type of bacteria. These germs can enter your body and live in your digestive tract. After many years, they can cause sores, called ulcers, in the lining of your stomach or the upper part of your small intestine. For some people, an infection can lead to stomach cancer.(3)(4). This study focuses on the identification of the phytochemical of *Azadirachta indica* responsible 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 *Azadirachta indica* contains Glutamic Acid, L-ascorbic acid, β-sitosterol, Oleic acid; Stigmasterol acid etc.It has already been established that *Azadirachta indica* plant belonging to Meliaceae 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 causeas 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 cinnamyl-alcohol dehydrogenase enzyme (protein database code 3TWO) is involved in various pathways (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 *Azadirachta indica* plant were downloaded from the website (refer). The protein database code of the cinnamyl-alcohol dehydrogenaseenzymewas identified from the website (refer). 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 cinnamyl-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 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 cinnamyl-alcohol dehydrogenase- Glutamic Acid interaction has the highest positive value of -CDOCKER energy (34.67) and minimum value of the difference (1.6337) between - C DOCKER interaction energy and - C DOCKER energy followed by Oleic acid & L-ascorbic acid. Thus the results indicated that and can effectively deactivate the cinnamyl-alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *Helicobacter*

pylori. Higher positive values for Glutamic Acid indicated that it was the most active ingredient against *Helicobacter pylori*. Thus, the key phytochemicals preventing ulcer caused by *Helicobacter pylori* are Glutamic Acid, L-ascorbic acid & Oleic acid.

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CONCLUSIONS

Formerly said that *Azadirachta indica* plant has medicinal action against ulcer,which is effected by *Helicobacter pylori.* This study was carried out to know the theoretical basis of this observation. For the identification of the phytochemical which are having quality of prevention, molecular docking operation was performed using Discovery studio module of Biovia software . The Phytochemicals (Glutamic Acid, L-ascorbic acid, β-sitosterol, Oleic acid, Stigmasterol) which can have a significant interaction with the vital enzyme (cinnamyl-alcohol dehydrogenase) of the microbe. The above operation done by molecular docking showed that Stigmasterol and β-sitosterol can not inhibit the metabolic pathway of respective micobe by deactivating the disease causing enzyme.The rest three Phytochemicals (Glutamic Acid, L-ascorbic acid& Oleic acid) form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe.Thus, this experiment could explain that the presence of Glutamic Acid, L-ascorbic acid & Oleic acid provided the medicinal values to *Azadirachta indica* against ulcer caused by *Helicobacter pylori.*

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Table 1. Results of Cdocking of Phytochemicals With Cinnamyl-Alcohol Dehydrogenase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Glutamic Acid	34.67	33.0363	1.6337
2	L-ascorbic acid	11.2294	28.9676	17.7382
3	β-sitosterol	FAILED	FAILED	NA
4	Oleic acid	31.3632	39.8618	8.4986
5	Stigmasterol	FAILED	FAILED	NA

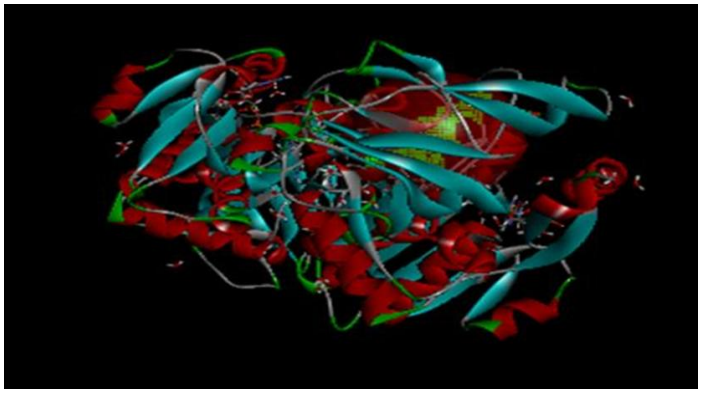

Figure 1. Active Site of Cinnamyl-Alcohol Dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Boswellia serrata* against Diarrhoea

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ABSTRACT

Phytochemical is a term that refers to a variety of plant derived compounds with therapeutic activity such as antiinflammatory, anticarcinogenic, antimutagenic, and antioxidant, considered to be beneficial to human health. It has been reported that *Boswellia serrata* 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 isglyceraldehyde-3-phosphate 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 possitive values of both the parameters indicated that out of different phytochemicals P-cymene and Boswellic acid can effectively deactivate the glyceraldehyde-3-phosphate dehydrogenase enzyme thereby interrupting the life cycle of *Campylobacter jejuni*.

Key Words: Phytochemical, Biovia, Discovery studio, *Boswellia serrata*.

INTRODUCTION

Life is a biological concept about the characteristics, state or mode that isolate a living thing from dead matter. Life was natural, difficult to survive, non-toxic and hygienic during ancient period. The modern life style has a number of advantages consisting of easing people life, saving hundred of peoples lives by new development of medicine and vaccines. In contrast modern life style patterns possess many negative effects on health physically, psychologically and socially. In modern time life is comfortable, readymade, streessfull and unhygienic. One of the main cause of unhealthy is to intake high amount of unhygienic foods. Risk of obesity is the main culprit of modern life style. Diabetes, heart disease, respiratory diseases, cancer and many other detrimental or harmful diseases are signs for life

destruction due to high intake of fatty foods. Plants have been used for medicinal purpose long before prehistoric period. Population rise, inadequate supply of drugs, prohibitive cost of treatements, side effects of several synthetic drugs and occurance of drug registive infectious diseases have led to emphasis on the use of plant materials as a source of medicines for a wide variety of human aliments (Zahid,2016). The medicinal value of the plants lies in some chemical substances that produce a definite physiological actions on the humanbody,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 and flowers, roots, fruits and seeds.Similarly *Boswellia serrata* plants also have phytochemicals,extracted from leaves . Oleo gum resin of bark extract having anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes, anti diarrhoeal, anti asthmatic, anti fungal, anti rheumatic, anti complementary and anti analgesic action (Iram,et.al.,2017). About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plants. In the last few years there has been an exponential growth in the field of herbal medicines (Fatima, et al., 2012). A little caution, small changes in lifestyle and care if taken, we can prevent these lifestyle related diseases from increasing.

Boswellia serrata belongs to family *Burseraceae*, which grows in dry mountain region of India, northen Africa and middle East. The family of *Burseraceae* is represented with 70 Genera and 600 species from them 25 known species belonging to Genus *Boswellia* (Siddiqui et al 2011). *Boswellia serrata* leaves extract is used to cure disease like diarrhoea (Maryam et al 2014). *Boswellia serrata* is known to contain phytochemicals like Boswellic acid, D-limonene, Incensole acetate, p-cymene, sabinene, Terpinen-4-ol etc(Sharma et al.2009). But the Boswellic acid contain various phytochemicals from them acetyl-11-keto-B-Boswellic acid is the most potent inhibitor of enzyme responsible for inflammation (Siddique et al. 2011). 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 *Campylobacter*g enerally cause diarrhoea, from them *Campylobacter jejuni* is another. They are spiral shapedgram negative bacteria. Natural reservoir of this bacteria is wild birds (Dasti et al.2009). According to United States Centers for Disease control,there are about 1.3 million cases of Campylobacter infection the condition is most common in children under 4 years old and young adult between 15 to 44 years old(Fischer et al.2019). Infection with *Campylobacter jejuni* results from the ingestion of contaminated food or water.

Humans become infected most frequently through contaminated water or food.The organism penetrates the gastrointestinal mucus , by high mortility and spiral shape body.The bacteria adheres to the gastro enterocytes and once adhered then induce diarrhoea by toxin release (Wallis et al.1994).This study focuses on the identification of the phytochemical of *Boswellia serrata* responsible to cure diarrhoea caused by *Campylobacter species.*

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 *Boswellia serrata* contains Boswellic acid, D-limonene, Incensole acetate, P-cymene, sabinene etc. It has already been established that *Boswellia serrata* plant belonging to *Burseraceae* 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 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 *Campylobacter jejuni.* It has been found that glyceraldehyde-3-phosphate dehydrogenase enzyme (protein database code 1B7G) is involved in glycolysis or gluconeogenesis, biosynthesis of secondary metabolites, carbon metabolism, biosynthesis of aminoacid, biosynthesis of antibiotics (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 Boswellia serrataplant were downloaded from the website (Pub-chem). The protein database code of the glyceraldehyde-3-phosphate 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 phytochemicals 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 Isocitrate 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 (Brinda,et.al.2019).

Table1 shows that glyceraldehyde-3-phosphate dehydrogenase P-cymene interaction has the highest possitive value of -CDOCKER energy (20.529) and minimum value of the difference (1.9457) between - C DOCKER interaction energy and - C DOCKER energy followed by boswellic acid. Thus the results indicated that p-cymene and boswellic acid can effectively deactivate the glyceraldehyde-3-phosphate dehydrogenase enzyme thereby interrupting the life cycle of *Campylobacter jejuni*. High positive values of P-cymene indicate that it is the most active ingradient against *Campylobacter sp*. On the other hand phytochemicals like D-limonene, Sabinene and terpinen-4-ol can deactivate the enzyme to a small extent (negative -C-dockor energy and positive -C-docker interaction energy). Where as Incensole acetate can not bind to the active side of enzyme, Thus the key phytochemical preventing diarrhoea by *Campylobacter jejuni is* P-cymeneand Boswellic acid.

CONCLUSION

It was previously known that *Boswellia serrata* plant has medicinal action against diarrhea. Diarrhoea is caused by *Campylobacter 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 (Boswellic acid,D-limonene,P-cymene,Incensole acetate,sabinene,Terpinen-4-ol), which can have a significant interaction with the vital enzyme (Glyceraldehyde-3-phosphate dehydrogenase) of the microbe. It was found that and P-cymene and Boswellic acid can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Other phytochemicals are not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of p-cymene and Boswellic acid phytochemicals provided the medicinal values to *Boswellia serrata* against diarrhoea caused by *Campylobacter jejuni.*

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Table 1. Results of CDocking of phytochemicals with Glyceraldehyde-3-phosphate dehydrogenase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	P-cymene	20.529	22.4747	1.9457
2	Boswellic acid	14.0048	25.8787	11.8739
3	Terpinen-4-ol	-6.32894	24.7613	31.09024
4	Sabinene	-12.8221	21.6368	34.4589
5	D-limonene	-21.4988	18.0224	39.5212
6	Incensole acetate	-337.116	-61.9394	275.1766

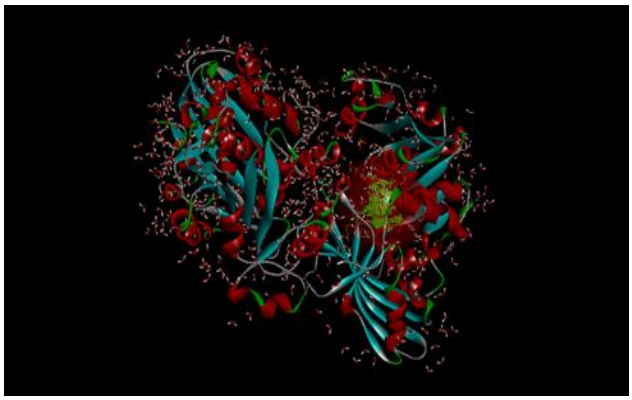

Figure: 1. Active site of glyceraldehyde -3-phosphate dehydrogenase

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Boswellia serrata* against Dysentery

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Boswellia serrata* plant extract is used to cure dysentery. The plant extract contains different phytochemicals. Dysentery is caused by *Entamoeba histolytica* species. One of the key enzyme 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 P-cymene and Boswellic acid can deactivate the Alcohol dehydrogenase enzyme thereby interrupting the life cycle of *Entamoeba histolytica.*

Key Words: phytochemical, Biovia, Discovery studio, *Boswellia serrata, Entamoeba histolytica.*

INTRODUCTION

In the antediluvian times, life was slow, a bit atrocious still natural and healthy. In contrast with the past, now life is hasty, quitecongenial, readymade but more anxious and unhealthy. Changes in work approach, less physicality, sedentary jobs, easy but pessimistic life, bad and unhygienic eating propensities has exposed us to some notorious and deleterious health hazards like blood pressure, diabetes, obesity etc. ''Prevention is better than cure'', subsequently we must concern regarding these little things and can refrain ourselves from those diseases. Nature has been a vast source of restorative agents from vedik era and an impressive number of current pharmaceutical products have been obtained from natural source. The medicinal value of the plants lies in some chemical substances that generates a certain physiological impact on the human body. These substances are called phytochemicals, which can be used for medicinal purposes. Plants based medicinal constituents can be derived from

any part of plant like bark, leaves, flowers, roots, fruits, and seeds (Verma et.al 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. (Hussain et.al2011). About 40% of the total medicinal consumption is attributed to traditional tribal medicine (Fatima et.al 2012). Which is predominantly based on plants due to their safety and cost effectiveness. 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 (Singh et.al 2015).

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 (Singhai et.al 2009). Study on 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.

Boswellia serrata belongs to family Burseraceae, which grows in dry mountain region of India, northern Africa and Middle East. The family of Burseraceae is represented with 70 genera and 600 species. From them 25 known species belonging to Genus Boswellia. The plant contains various phytochemicals from them acetyl 11 keto -beta- boswellic acid is the most potent inhibitor of enzyme responsible for inflammation (Siddiqui et.al 2011). The leaves extract of *Boswellia serrata* contains phytochemicals but, Oleogum resin of the plant has been popularly used as traditional Chinese medicines to alleviate avariety of diseases(Ahmed et.al 2015).The plant contains phytochemicals like Boswellic acid, D-limonene, Incensole acetate, p-cymene, sabinene, Terpinen-4-ol etc. (Sharma et.al 2019). There is more possibility that these phytochemicals play a major role in curing dysentery. However, there is no report identifying the specific phytochemical responsible to cure dysentery.

Generally dysentery is of two types like Bacillary or shigellosis and amoebic dysentery. The bacillary infection is caused by bacteria *Bacillus shigella* and amoebic infection by *Entamoeba histolytica* a non-flagellated Protozoan parasite (Shirley et.al 2019). *E.histolytica* is the etiological agent of amoebiasis in humans. It exists in two form the Trophozoite which is the active, dividing form and the cyst is dermant and can survive for prolonged periods outside the host. Infection usually begins with ingestion of the cyst present in food or water contaminated with human fecal matter. The cyst can survive in the stomach and enter into the intestine, now cyst undergoes excystment and give rise to trophozoits which are multiply in the colon. Most of the times they live commensal to intestine but sometimes it attack the intestinal mucosa and causes dysentery (Sehgal et.al 1996). Dysentery is a febrile infection leading to frequent defecation with blood, pus, mucus in the stool (Shanks et.al (2016). The best estimate suggest that probably 480 million people were infected with *E.histolytica* and 36 million developed disabiling colitis or external abscesses in 1981 (Walsh et.al 1986). This study focuses on the identification of the phytochemical of *Boswellia serrata* responsible to cure dysentery caused by *Entamoeba histolytica.*

MATERIALS AND METHODOLOGY

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 *Boswellia serrata* contains boswellic acid, D-limonene, Incensole acetate, P-cymene, sabinene, Terpinen-4-ol etc. It has already been established that *Boswellia serrata* plant 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 cause as a result of *Entamoeba sp.* 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.histolytica*. It has been found that alcohol dehydrogenase enzyme (protein database code 1Y9A) is involved in different metabolism like Tryptophan metabolism, Phenylalanine metabolism,Valine metabolism,Methionine metabolism, Ethanol formation, Propanol degradation (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, which 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 *Boswellia serrata* plant were downloaded from the website (Pub-Chem). The protein database code of the alcohol 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 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 [Brinda,et.al.(2019)].

Table 1 shows that alcohol dehydrogenase P-cymene interaction has the highest value of -CDOCKER energy (12.4406) and minimum value of the difference (0.4821) between - CDOCKER interaction energy and - CDOCKER energy followed by boswellic acid. Thus the results indicated that P-cymene and Boswellic acid can deactivate the alcohol dehydrogenase enzyme there by interrupting the biological life cycle of *E. histolytica*. Higher positive value of P-cymene indicate that it is the most active ingredient against the microbe. Onthe Other hand D-limonene,

Incensoleacetate, Sabinene and terpinen-4-ol can deactivate the enzyme to a small extent (negative -C-dockor energy and positive -C-docker interaction energy) by bind to its activeside, Thus the key phytochemicals preventing dysentery caused by *E.histolytica* is P-cymene and Boswellic acid.

CONCLUSION

It was previously known that *Boswellia serrata* plant has medicinal action against dysentery. Dysentery is caused by *E. 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 (Boswellic acid, D-limonene, P-cymene,Incensole acetate,sabinene, Terpinen-4-ol), which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe. It was found that P-cymene and Boswellic acid can form strong bond with the enzyme there by inhibiting the metabolic cycle of the microbe. Other phytochemicals are not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of P-cymene and Boswellic acid phytochemicals provide medicinal value to *Boswellia serrata* against *E.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	P-cymene	12.4406	12.9227	0.4821
2	Boswellic acid	6.07175	15.5173	9.44555
3	Terpinen-4-ol	-13.4773	15.3683	28.8456
4	Sabinene	-17.4056	13.9505	31.3561
5	D-limonene	-24.0287	14.2101	38.2388
6	Incensole acetate	-38.1204	24.839	62.9594

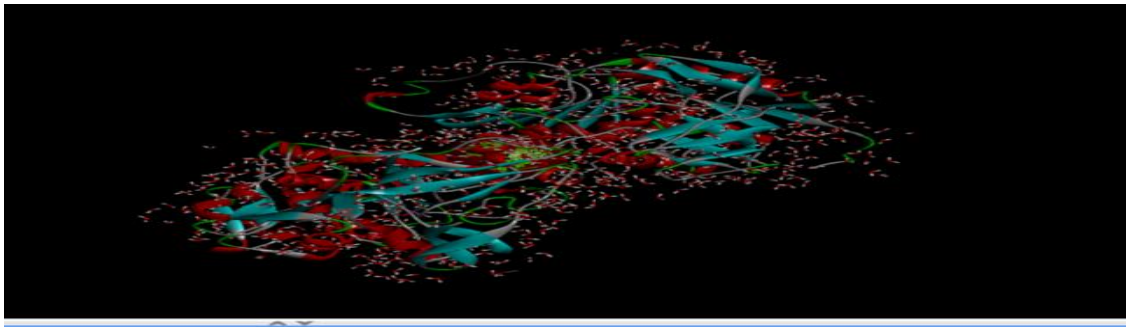

Fig. 1.Active Side Of Alcohol Dehydrogenase *Entamoeba histolytica*

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Flaxseed against Bronchitis

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ABSTRACT

Phytochemicals (phyto word derive from Gk word plant) are the chemical compounds having biologically active and naturally occurring compounds, which derives from the plants.They prevents the plant disease and damage and provide the color, aroma and flavor to the plants. In roots, stems, leaves, flowes, fruits and seeds of plant phytochemicals are collected. [1] It has been reported that *Flaxseed* plant extract is used to cure bronchitis. The plant extract contains different phytochemicals. Bronchitis is caused by *Streptococcus pneunonia.* One of the key enzymes involved in its biochemical pathwayisissite-specific DNA-Methyltransferase. 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 p-coumaric acid and valinic acidcan effectively deactivate the site-specific DNA-methyltransferase enzyme thereby interrupting the life cycle of *Streptococcus pneumoniae.*

Key Words: Phytochemical, Biovia, Discovery studio, *Flaxseed*.

INTRODUCTION

As long as the people search for drugs in nature to release their diseases.The uses of medicinal plants were intuitive from the beginning. Eventually the justification ofparticular medicinal plants for treatment of certain diseases were discovered; therefore the medicinal plants usage gradually abandoned the empiric framework and became founded on explicatory facts. [2]. As stated by economically, demographically and epidemiologiacially ;in terms of health now India is in a alteration state. Also India has creates an amelioration over the past deacades, in health section.By declining as is the rate of disease incidence, the life anticipation has crossed 67 years. Polio, guinea worm disease, yaws, and tetanus like diseases have been eradicated.[3]

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. 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.

Flaxseed belongs to familyLinaceae .It is a annual herb having blue coloured flower that produces small flat seeds which differs from golden yellow to reddish brown colour .To explain flax when consumed by humans, while Linseed shows that it is used for industrial purposes. Humans taken it since the ancient time and it is cultivated in more than 50 countries for various purposes such as for fiber, for medicinal product and for nutritional product. Due to the versatile uses of flaxseed, it relishes a better position among oil seeds. [4] Flaxseed leaves extract is used to cure disease like bronchitis (reference). *Flaxseed* is known to contain phytochemicals likeβ-sitosterol, ferulic acid, linamarin, caffeic acid, salisilic acid, sinapic acid, stigmatic acid, syringic acid, valilinic acid, p-coumaric acidetc. (reference). 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 genus *Streptococcus pneumoniae* generally cause bronchitis. They are gram positive bacteria in the shape of slightly pointed cocci. *Streptococcus pneumoniae* infection is a common bacterial disease that causes ear infection, lungs infection, blood stream infection. *Streptococcus pneumoniae* bacteria found in human upper respiratory tract. This study focuses on the identification of the phytochemical of *Flaxseed* responsible to cure bronchitis 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 βsitosterol, ferulic acid, linamarin, caffeic acid, stigmatic acid, syringic acid, valilinic acid, p-coumaric acid etc.It has already been established that *Flaxseed* plant belonging to family Linaceae 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 *Streptococcus pneumoniae*

It has been reported that bronchitis 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 site-specific DNA-methyltransferase (protein database code 2DPM) 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 *Flaxseed* plant were downloaded from the website (refer). The protein database code of the (2DPM) enzyme was identified from the website (refer). 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 site-specific DNA methyl transferase 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 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 site-specific methyl transferase-vanilic acid interaction has the highest positive value of -CDOCKER energy (14.1469) and minimum value of the difference (1.5756) between - C DOCKER interaction energy and - C DOCKER energy followed by. Thus the results indicated that p-coumaric and vanillic acidcan effectively deactivate the alanine racemase *Streptococcus pneumoniae* 3S46 enzyme thereby interrupting the biological cycle of *streptococcus pneumoniae sp.* Higher positive values for p-coumaric indicated that it was the most active ingredient against *Streptococcus pneumoniae sp.* On the other hand, salisilic acid, syringic acid, ferulic acid deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy). Caffeic acid and β-sito acid cannot interact with (site-specific methyl DNA Transferase) enzyme. Thus, the key phytochemicals preventing bronchitis caused by *Streptococcus pneumoniae* are p-coumaric and vanilic acid.

CONCLUSIONS

It was previously known that *Flaxseed* plant has medicinal action against bronchitis. Bronchitis 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 (ferulic acid, stigmatic acid, caffeic acid, linamain, β-sitosterol, sinapic acid, valinic acid, p-coumaric), which can have a significant interaction with the vital enzyme (site specific methyl DNA transferase) of the microbe. It was found that p-coumaric and vanilic acid) can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Salilcylic acid, syringic acid were found to be not much effective in deactivating the enzyme of the microbe. Caffeic acid and sinapic acid cannot deactivate the enzyme. Thus, this study could explain that the presence of p-coumaric and vanilic acid provided the medicinal values to *Flaxseed* against bronchitis caused by *Streptococcus pneumoniae Sp.*

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Table 1. Results of Cdocking of Phytochemicals Withsite-Specific Methyl DNA Transferase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	p-coumaric	14.1469	15.7225	1.5756
2	Salisilic acid	11.1097	18.1759	7.0698
3	Syringicacid	-997.628	-180.461	817.167
4	Vanilic acid	14.6339	16.8227	2.1888
5	Ferulic acid	10.9004	13.8273	2.9269
6	Caffeic acid	failed	Failed	NA
7	Beta- sitos	failed	Failed	NA
8	Sinapic acid	failed	Failed	NA

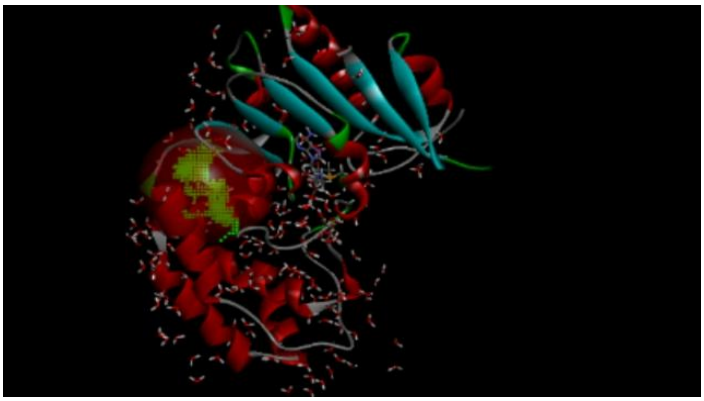

Figure 1.Active site of (site-specific DNA methyl tramsferase)enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Nardostachys jatamansi* against *Candida tropicalis* Causing Candidiasis

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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 candidiasis. The plant extract contains different phytochemicals. Candidiasis is caused by *Candida tropicalis*. One of the key enzymes involved in its biochemical pathway is acetoacetyl CoA 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 values for both the parameters indicated that out of different phytochemicals 1-octacosanal can effectively deactivate the enzyme thereby interrupting the life cycle of *Candida tropicalis.*

Keywords: Phytochemical, Biovia, Discovery studio, *Nardostachys jatamansi*, *Candida tropicalis.*

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 [1]. 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 [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]. 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. [4].

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. [5]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Jatamansi belongs to family Caprifoliaceae. Methanolic extract is used to cure disease like candidiasis. *Jatamansi* is known to contain phytochemicals like 1-octancosanol, beta-sitosterol, oleanolic acid,ursolic acid etc. *Candia* normally lies on the skin and inside the body, in place such as the mouth, throat, gut and vagina without causing any problem. Oral thrush, candidiasis that develops in mouth or throat is called thrush.Tiredness and fatigue, recurring genital or urinary tract infections, digestiveissue, Sinus infection, joint pains are some of the most common symptoms for the infection. There is high possibility that these phytochemicals play a major role in curing candidiasis. However, there is no report identifying the specific phytochemical responsible to cure candidiasis. A group of yeast/fungus belonging to family *Saccharomycetaceae* generally cause candidiasis. Candida appears as gram +ve. Budding yeast cell (*blastoconidia*) and/or pseudohyphae showing regular points of construction. This study focuses on the identification of the phytochemical of *Nardostachy jatamansi* responsible to cure candidiasis caused by *Candida tropicalis* yeast (a type of fungus).

METERIALS 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 *Nardostachy jatamansi* contains 1-octacosanol, beta-sitosterol, oleanolic acid, ursolic acid, etc. It has already been established that plant *Nardostachy jatamansi* belonging to Caprifoliaceae family has potential to help controlling candidiasis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling candidiasis.

Enzyme Found in *Helicobacter pylori*

It has been reported that candidiasis can cause as a result of *Candida tropicalis* 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 candida tropicalis. It has been found that acetoacetyl CoA reductase (protein database code 3GK3) is involved in glyoxylate, dicarboxylate 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 *Nadrostachys jatamansi* plant were downloaded from the website [6]. The protein database code of 3GK3 the enzyme was identified from the website [7]. 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 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 [8].

Table 1 shows that eacetoacetyl-CoA reductase – 1-octacosanol interaction has the highest positive value of -CDOCKER energy 64.0539 and minimum value of the difference 11.388 between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that 1-octacosanol can effectively deactivate the enzyme ecetoacetyl-CoA reductase thereby interrupting the biological cycle of *Candida tropicalis.* Higher positive values for indicated that it was the most active ingredient against candida tropicalis. On the other hand beta-sitosterol, oleanolic acid, urasolic acid, hinokinin can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing candidiasis caused by *Candida tropicalis* are acetoacetyl CoA reductase.

CONCLUSIONS

It was previously known that *Nardostachy jatamansi* plant has medicinal action against candidiasis. Candidiasis is caused by *Candida tropicalis*. 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, beta-sitosterol, oleanolic acid, ursolic acid, which can have a significant interaction with the vital enzyme eacetoacetyl-CoA reductase of the microbe. It was found that 1-octacosanal can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Beta-sitosterol, Oleanolic acid, urasolic acid, hinokinin, were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of 1-octacosanol provided the medicinal values to against *Nardostachy jatamnsi* caused by *Candida tropicalis.*

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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	1-Octacosanol	64.0539	75.4419	11.388
2	Beta-Sitosterol	-40.0336	51.6158	91.6494
3	Oleanolic Acid	-140.088	21.3579	161.4459
4	Urasolic Acid	-134.109	18.2213	152.3303
5	Hinokinin	-0. O920676	47.1681	47.2601676

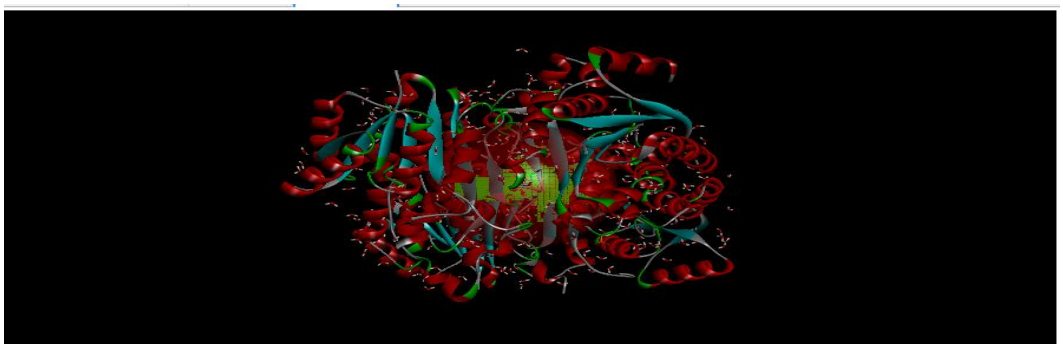

Figure 1. Active site of acetoacetyl CoA reductase enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Hedychium spicatum* against *Escherichia coli* Causing Diarrhea

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ABSTRACT

Diarrhea is a common bacterial infection of intestinal tract. It is generally a short-lived condition but if remain persistent it may indicate some serious issues. Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Hedychium spicatum* plant extract is used to cure Diarrhea. The plant extract contains different phytochemicals. Diarrhea is caused by *Escherichia coli.* One of the key enzymes involved in its biochemical pathway is alcohol dehydrogenase. The molecular docking of the phytochemicals with the microbial 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 3-hydroxy Gama eudesmal-1 can effectively deactivate the enzyme thereby interrupting the life cycle of *E. coli.*

Key Words: Phytochemical, Biovia, Discovery studio, *Hedychium spicatum, Escherichia coli*

INTRODUCTION

Diarrhea is a common disease which is directly linked with bad hygienic habits, changed dietary regime and lack of fibrous nutrition in an individual's meal plan. Sometimes it's a common symptom among some serious illness. 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 [1]. 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.[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] 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 and cost effective. 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 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.[4] Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Kapoor kachri belongs to family Zingiberaceae. Kapoor kachri is a plant species native to China (Guizhou, Sichuan, Tibet, Yunnan), the Himalayas, Myanmar, Thailand and Ethiopia. *Hedychium spicatum* is a small, hardy perennial that grows to around 1 m, with green leaves and large orange and white flowers. It is also commonly known as spiked ginger lily, or perfume ginger. [5] Kapoor kachri leaves extract is used to cure disease like Diarrhoea. Kapoor kachri is known to contain phytochemicals like Beta sitisterol, Borneol 1, Camphor, Beta carotene, 3 hydeoxy Gama eudesmal 1, 9 hydroxy hedychenone etc. 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. They are Gram-negative, facultative anaerobic, rod-shaped, coliform bacteria. Commonly found in the lower intestine of warm-blooded organisms. *E. coli* infection is a common bacterial disease that affects the intestinal tract. The bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food [6] However, this infection can be kept in check by inhibiting the metabolic cycle of the microbe. Alcohol dehydrogenase enzyme of the *E.Coli* bacterium is targeted by the phytochemicals of the plant to inhibit the ethanol fermentation pathway resulting in controlling of the disease. This study focuses on the identification of the phytochemical of *Hedychium spicatum* 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.

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 *Hedychium spicatum* contains Beta sitisterol, Borneol-1, Camphor, Beta carotene, 3-hydroxy Gama eudesmal-1, 9-hydroxy hedychenone etc. It has already been established that *Hedychium spicatum* plant belonging to Zingiberaceae family has potential to help controlling Diarrhea. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of Diarrhea.

Enzyme Found in *Escherichia coli*

It has been reported that diarrhea 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 alcohol dehydrogenase enzyme (protein database code 4GKB) is involved in ethanol fermentation (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 perform molecular docking. In this process first the sdf files for the phytochemicals found in the *Hedychium spicatum* plant were downloaded from the website. [7] The protein database code of the alcohol dehydrogenase enzyme was identified from the website. [8] 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 [9]. Table 1 shows that (3-hydroxy Gama eudesmal-1)-(alcohol dehydrogenase) interaction has the highest positive value of -CDOCKER energy (11.0831) and minimum value of the difference (1.9377) between - C DOCKER interaction energy and - C DOCKER energy followed by 9-hydroxy hedychenone. Thus, the results indicated that 3-hydeoxy Gama eudesmal-1 can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *Escherichia coli*. Higher positive values for 3-hydeoxy Gama eudesmal-1 indicated that it was the most active ingredient against *Escherichia coli*. On the other hand, Borneol-1, Beta carotene, Beta sitisterol can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Camphor cannot interact with alcohol dehydrogenase enzyme. Thus, the key phytochemicals preventing diarrhea caused by *Escherichia coli* are 3-hydroxy Gama eudesmal-1.

CONCLUSION

It was previously known that *Hedychium spicatum* 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 Beta sitisterol, Borneol-1, Camphor, Beta carotene, 3-hydroxy Gama eudesmal-1, 9-hydroxy hedychenone, which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe. It was found that 3-hydroxy Gama eudesmal-1 can form strong bond with the enzyme followed by 9-hydroxy hedychenone, successfully inhibiting the metabolic cycle of the microbe. Borneol-1, Beta carotene, Beta sitisterol, were found to be not much effective in deactivating the enzyme of the microbe. Camphor cannot deactivate the enzyme. Thus, this study could explain that the presence of 3-hydeoxy Gama eudesmal-1 provided the medicinal values to *Hedychium spicatum* against Diarrhea 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	3-HYDROXY GAMA EUDESMAL-1,	11.0831	13.0208	1.9377
2	9-HYDROXY HEDYCHENONE	6.29307	8.47561	2.18254
3	BORNEOL-1,	-37.8047	16.1205	53.9252
4	BETA CAROTENE,	-80.3597	36.0345	116.3942
5	BETA SITISTEROL,	-45.8863	35.9004	81.7867
6	CAMPHOR,	FAILED	FAILED	FAILED

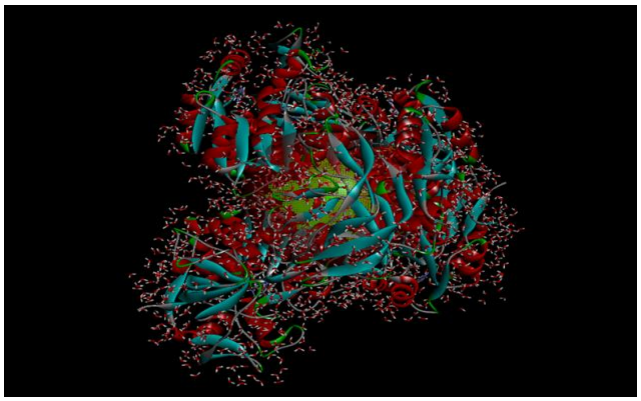

Figure 1 : Active Site of Alcoholdehydrogenase Enzyme

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RESEARCH ARTICLE

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

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *lagerstroemia spaciosa* plant extract is used to cure malaria. The plant extract contains different phytochemicals. Malaria is caused by *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium* malariae,. One of the key enzymes involved in its biochemical pathway is Lactate dehydrogenase. The Molecular docking of the phytochemicals with the enzymes was studied using Bio via 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 effectively deactivate the lactate dehydrogenase enzyme thereby interrupting the life cycle of malaria.

Key Words: Phytochemical, Biovia Discovery studio, *Lagerstroemia speciosa, Plasmodium*.

INTRODUCTION

In olden days, life was natural, slow, difficult at times but healthy. Today, in modern times, life 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 disease. With tens of thousands of plant species on earth, We are endowed with an enormous wealth of medicinal remedies from mother nature (Pan et al.2013). 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 (Pattanayak et al.2010). 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 et al.2011).Medicinal plants play a key role in human health care. According to WHO over 80% of the world population relies on the use of traditional medicine (Gopal et al. 2014), which is predominantly based on plants, the reasons of this popularity are the safety, efficacy of medicinal plants and their cost effectiveness. Many of the medicinal plants are used as spices and food items. They also played an important role to produce many medicines like herbal medicine, alternative medicine, homoeopathy but less allopathy medicines (Jawla et al.2009). 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 (Gopal et al.2014). 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 (Tiwari et al.2017). Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention (Andino et al 2015*).*

Lagerstroemia speciosa is native to Asia-tropical and subtropical region. *Lagerstroemia* belongs to family lythraceae. Poultice of the *Lagerstroemia speciosa* leaf is used to cure disease like malaria (Snafi et al.2019) . The phytochemical investigation of *Lagerstroemia speciosa* leaf and fruit revealed that it contains Alkaloids, Alfa amino acids, Glucocorticoids, Glycosides, Saponins, Tannins etc (Snafi et al.2019). *Lagerstroemia speciosa* possessed many pharmacological effects included antimicrobial, antioxidant, anticancer, antidiabetic and anti-inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, central nervous, inhivition of xanthine oxidase, hepatoprotective and nephroprotective effects (snafi et al.2019). There is high possibility that these phytochemicals play a major role in curing Malaria. However, there is no report identifying the specific phytochemical responsible to cure malaria

A group of protozoa belonging to genus *Plasmodium* generally cause malaria. From them *P.falciparum* is the most dangerous. Uninucleate sporozoits in the salivary gland of female anophelies mosquito injected into human host when it feeds and circulate through bloodstream and quickly invade liver cell and then infect to the RBC or host erythrocyte by showing symptoms of fever, sweats, chills, headache, muscular pain, weakness, vomiting, cough, diarrhoea, abdominal pain.Further sexual lifecycle continues in the mosquito gut.(Crutcher et al.1996).About 2 million confirmed malaria cases and 1,000 deaths are reported annualy,although 15 million cases and 20,000 deaths are estimated by WHO South east Asia.(Kumar et al 2007). This study focuses on the identification of the phytochemical of *Lagerstroemia speciosa* responsible to cure malaria caused by *plasmodium 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 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, Alfa amino acids, Glucocorticoids, Glycosides, Saponins, Sitosterols, Tannins etc. It has already been established that *Lagerstroemia speciosa* belonging to Laurels family has potential to help controlling malaria. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of malaria .

Enzyme found in *Plasmodium falciparum*

It has been reported that malaria can cause as a result of *plasmodium* sp. 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 *plasmodium* sp. parasite. It has been found that lactate dehydrogenase enzyme (protein database code 1T2E) is involved in different metabolism, like Glycolysis and Gluconeogenesis (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 parasitic 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 *Lagerstroemia speciosa* plant were downloaded from the website (Pub-chem). The protein database code of the lactate 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 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. C-DOCKER 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 [Brinda et al 2019]. Table 1 shows that lactate dehydrogenase-alpha aminoacid interaction has the highest positive value of -CDOCKER energy (26.8997) and minimum value of the difference (2.2731) between - C DOCKER interaction energy and - C DOCKER energy followed by alpha amino acid. Thus the results indicated that saponins, glycosides and alpha amino acid can effectively deactivate the lactate dehydrogenase enzyme thereby interrupting the biological cycle of *Plasmodium falciparum.* Higher positive values for Alpha amino acid indicated that it was the most active ingradient against *Plasmodium sp*. On the other hand, Glucocorticoids, Alkaloids, Sitosterols, Tannins cannot interact with Lactate dehydrogenase enzyme. Thus the key Phytochemicals preventing malaria caused by *Plasmodium falciparum* are Saponins, glycosides and alpha amino acids.

CONCLUSION

It was previously known that *Lagerstroemia speciosa* plant has medicinal action against malaria . Malaria is caused by *plasmodium.* 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 acid, Glucocorticoids, Glycosides, Saponins, Sitosterols, Tannins), which can have a significant interaction with the vital enzyme (Lactate dehydrogenase) of the microbe. It was found that Saponins, Glycosides and Alpha amino acids can form strong bond with the enzyme successfully. Thus, this study could explain that the presence of Saponins, Alpha aminoacids and Glycosides provided the medicinal values against malaria caused by *plasmodium sp*.

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

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference BETWWEN - C DOCKER INTERACTIONENERGY AND - C DOCKER ENERGY
1	Alpha aminoacid	26.8997	23.3872	3.5125
2	Glycosides	24.6768	29.6374	4.9606
3	Saponins	11.7589	14.032	2.2731
4	Glucocorticoids	Failed	Failed	NA
5	Alkaloids	Failed	Failed	NA
6	Sitosterols	Failed	Failed	NA
7	Tannins	Failed	Failed	NA

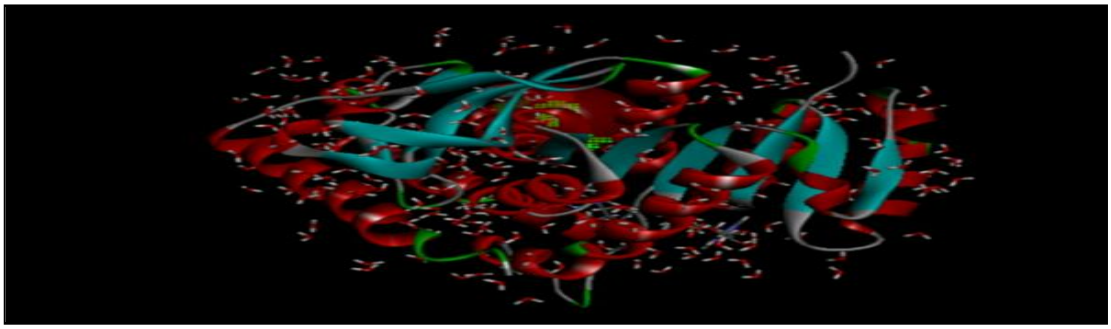

Fig.1.Active side of lactate dehydrogenase *Plasmodium falciparum*

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from Malkangni (Celastrus *paniculatus*) against Glutamate Racemase of *Streptococcus pyogenes* Causing Throat Infection

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ABSTRACT

Phytochemicals are precious for humans. Phytochemical is a term that refers to a variety of plant – derived compounds with therapeutic activities such as antti- carcinogenic, anti- inflammatory, anti-mutagenic, anti-oxidant properties (McGuire 2011). It has been reported that Malkangni plant extract is used to cure throat infection.The plant extract contains different phytochemicals. Throat infection is caused by *Streptococcus pyogenes*. One of the key enzymes involved in its biochemical pathway is *Glutamate racemase*. 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 interactionan energy. High positive values for both the parameters indicated that out of different phytochemical paraffinic hydrocarbon (benzene) can effectively deactivate the *Glutamate racemase* enzyme thereby interrupting the life cycle of streptococcus pyogenes.

Keywords: Phytochemicals, BIOVIA, Discovery studio, *Celastrus paniculatus, Streptococcus pyogenes.*

INTRODUCTION

Lifestyle diseases characterize those diseases whose occurrence is primarily based on the daily habits of people and are a result of an inappropriate relationship of people with their environment. The main factors contributing to lifestyle diseases include bad food habits, wrong body posture, and disturbed biological clock (Yuan H; NCBI). People are predisposed to various diseases based on their way of living occupational habits. They are preventable and can be lowered with changes in diet, lifestyle and environment (NCBI). As a discipline, preventive medicine has traditionally been described to encompass primary, secondary and tertiary prevention. The fields of preventive

medicine and public health share the objectives of promoting general health, preventing disease and applying epidemiologic techniques to these goals (AJPM).

Natural products, which have evolved over millions of years, have a unique chemical diversity, which results in diversity in their biological activities and drug- like properties. Those products have become one of the most important resources for developing new lead compounds and scaffolds. Natural products will undergo continual use toward meeting the urgent need to develop effective drugs and they will play a leading role in the discovery of drugs for treating human diseases (Nhp. Gov.in). Historically, natural products have been used since ancient times and in folklore for the treatment of many diseases and illness. Natural products (secondary metabolites) have been used the most successful source of potential drugs. Nevertheless, natural products continue to unique structural diversity in comparison to standard combinatorial chemistry, which presents opportunities for discovering mainly novel low molecular weight lead compounds await discovery with the challenge being how to access this natural chemical diversity (NCBI).

Traditional medicinal practices have formed the basis of most of the early medicines followed by subsequent clinical, pharmacological and chemical studies. Probably the most famous and well known example to date would be the synthesis of the anti- inflammatory agent, salicin isolated from the bark of the willow tree *salix alba* . (NCBI). The term "medicinal plant" include various types of plant used in herbalism. It is the use of plants for medicinal purposes. Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have lead to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human aliments.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that , use of herb treatments is independent of any age groups and the sexes. The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. The herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure. (NHP). Phytochemicals are a powerful group of chemicals that are derived from natural resources especially with plant origin. They have shown to exhibit chemoprevention and chemotherapeutic effects not only in cell lines and in animal models of cancer but some of them are in clinical trial phase (sincedirect.com)

Medicinal plants such as Aloe, Tulsi, Neem, Turmeric and Ginger cure several common aliments. These are considered as home remedies in many parts of the country. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial or synthetic drugs. A part from that , these plants play a critical role in the development of human cultures around the whole world. Now a day medicinal herbs are important sources for pharmaceutical manufacturing. Recipes for the treatment of common aliments such as diarrhea, constipation, hypertension, low sperm count, dysentery and weak penile erection, piles, coated tongue, menstrual disorders, leucorrhoea nand fevers are given by the traditional medicine practitioners very effectively (NHP). Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, safflower are used to heal wounds sores and boils. Many herbs are used as blood purifiers to alter or change a long – standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever. Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds. Ginger and cloves are

used in certain cough syrups. Certain medicinal herbs have disinfectant property, which destroys disease causing germs. They also inhibit the growth of pathogenic microbes that cause communicable diseases (NHP).

Brahmi, *Bacopa monnieri* is a nootropic ayurvedic herb known to be effective in neurological disorder from ancient times. Numerous approaches including natural and synthetic compounds have been applied against Alzheimer's diseases. Amyloid beta and tau are the hallmarks proteins of several neuronal disfunctions resulting in Alzheimer's diseases (T. Dubey ; sincedirect). There are a number of reasons to think that HMP (Herbal Medicinal Product) have a potential to become a significant part of efforts to advance drug discovery and development. Looking forward, a good practices and a paradigm change are necessary to study HMP (Herbal Medicinal Product) in a productive way (NCBI).

These herbal products are today the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment. Although, herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetic drugs is over and people are returning to the naturals with hope of safety and security. It's time to promote them globally (NHP). As our lifestyle is now getting techno- savy, we are moving away from nature. While we cannot escape from nature because we are part of nature. As herbs are natural products that are free from side effects, they are comparatively safe, eco- friendly and locally available(NHP).

Malkangni (*Celastrus panicuculatus*) is also known as staff tree. Malkangni belongs to family Celastraceae. Malkangni leaves extract is used to cure disease like Throat infection (reference). Malkangni is known to contain phytochemicals like cardiac glycosides (calatropin), diclofenac glycosides (anthraquinone), glycosides, paraffinic hydrocarbon (benzene), piroxicametc (reference).There is high possibility that these phytochemicals play a major role in curing Throat infection. However, there is no report identifying the specific phytochemical responsible to cure throat infection. A group of bacteria belonging to genus *Streptococcus* generally cause throat infection. These bacteria are extracellular and made up of non motile and non sporing cocci. It is a gram positive coccus. *Streptococcus pyogenes* is a bacterium that can be found on the skin or in the respiratory tract. The bacteria are spread by direct contact with nose and throat discharges of an infected individual. *Streptococcus pyogenes* is an important global human pathogen that causes a wide variety of acute infection such as pharyngitis (Mandell; 2015). Streptococcal bacteria are highly contagious. They can spread through airborne droplets when someone with the infection coughs or sneezes or through shared food or drinks (Myoclinic.org). This study focouses on the identification of the phytochemical of Malkagni responsible to cure throat infection caused by *Streptococcus pyogenes. Streptococcus pyogenes* contain different enzymes like UDP – glucose, IMP – dehydrogenase, shikimate dehydrogenase. My referring enzyme is glutamate racemase.

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 Malkangni contains cardiac glycosides (calatropin), diclofenac, glycosides

(sucrose), glycoside (anthraquinone), paraffinic hydrocarbon (benzene), piroxica etc.It has already been established that Malkangni plant belonging to Celastraceae family has potential to help controlling throat infection.. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of throat infection.

Enzyme Found in *Streptococcus pyogenes*

It has been reported that Throat infection can causeas a result of *Streptococcus pyogene* 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 pyogenes* bacteria. It has been found that 8 CBM/c \][p0o9i8u7ytr+\][lutamate racemase enzyme (protein database code 2OHV) is involved in/ vc+=--09886543dfj ';lkkjj k *87\][phenyl D- glutamate and D- glutamine metabolism (KEGG) and very crucial for survival of the articular 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 Malkangni plant were downloaded from the website (refer). The protein database code of the *Glutamate racemase* enzymewas identified from the website (refer). 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 *Glutamate racemase* 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 *Glutamate racemase*- paraffinic hydrocarbon interaction has the highest positive value of -CDOCKER energy (7.70763) and minimum value of the difference (2.13473) between C DOCKER interaction energy and C DOCKER energy followed by . Thus the results indicated that paraffinic hydrocarbon can effectively deactivate the shikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Streptococcus pyogenes*._Higher positive values for paraffinic hydrocarbon indicated that it was the most active ingredient against *Streptococcus pypogenes.* On the other hand, cardiac glycosides (calatropin), diclofenac, glycoside (anthraquinone), glycosides (sucrose), piroxicam cannot interact with *Glutamate racemase* enzyme. Thus, the key phytochemical preventing throat infection caused by *Streptococcus pyogenes* is paraffinic hydrocarbon hydrocarbon (benzene).

CONCLUSION

It was previously known that Malkangni plant has medicinal action against throat infection. Throat infection is caused by *Streptococcus pyogenes*. 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 (anthraquinone, diclofenac, piroxicam, calatropin and paraffinic hydrocarbon) which can have a significant interaction with the vital enzyme (*Glutamate racemase*) of the microbe.It was found that paraffinic hydrocarbon (benzene) can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Cardiac glycosides, diclofenac, anthraquinone glycosides (sugar) , piroxicam cannot deactivate the enzyme. Thus, this study could explain that the presence of paraffinic hydrocarbon (benzene) provided the medicinal values to malkangni against throat infection caused by *Streptococcus pyogenes.* This article brings to light in the silico analysis of phytochemical from *Celastrus paniculatus ag*ainst throat infection.

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Table 1. Results of CDocking of phytochemicals with Glutamate racemase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN - C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Paraffinic hydrocarbon (Benzene)	7.70763	9.8423	2.13473
2	Cardiac glycosides (calatropin)	Failed	Failed	Failed
3	Diclofenac	Failed	Failed	Failed
4	Glycosides	Failed	Failed	Failed
5	Glycosides (Anthraquinone)	Failed	Failed	Failed
6	piroxicam	Failed	Failed	Failed

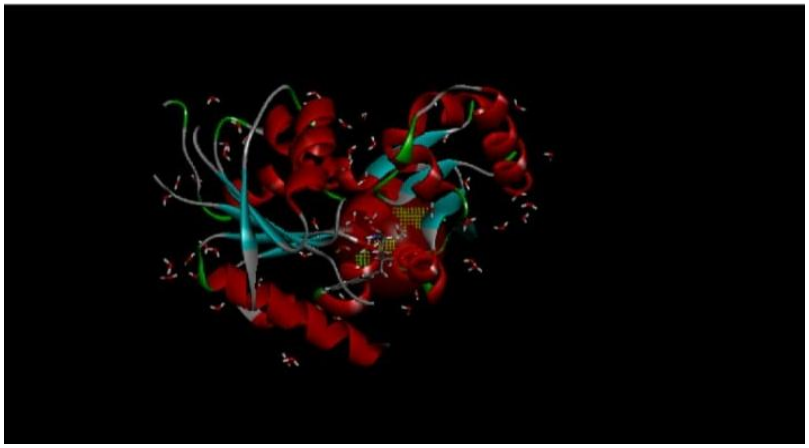

Figure 1. Active site of *Glutamate racemase* enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Rauvolfia serpentina* against Enoyl-[Acyl-Carrier Protein] Reductase of *Vibrio cholerae* Causing Cholera

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ABSTRACT

Cholera, caused by the infection of toxigenic *Vibrio cholerae* to humans. Currently, cholera has been becoming endemic in an increasing number of geographical areas, reflecting a failure in implementation of control measures. Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Rauvolfia serpentine* plant extract is used to cure cholera. The plant extract contains different phytochemical is caused by *Rauvolfia sp*. One of the key enzymes involved in its biochemical pathway is Enoyl-[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 values for both the parameters indicated that out of different phytochemicals some can effectively deactivate the Enoyl-[acyl-carrier protein] reductase enzyme thereby interrupting the life cycle of *Vibrio cholerae.*

Key Words: Phytochemical, Biovia, Discovery studio, Rauvolfia serpentina, *Vibrio cholerae*

INTRODUCTION

Cholera, an ancient and devastating acute diarrheal illness, is caused due to toxigenic *Vibrio cholerae* infection to humans (both adults and children), and currently is a serious global problem. The disease causes profuse watery diarrhea and can quickly lead to severe dehydration and death [1]. The World Health Organization (WHO) describes cholera as a global threat to public health and one of the key indicators of social development, stating that with the increased reporting of cholera in 2006, almost every developing country is with an outbreak or the threatof an epidemic [2].

In ancient days, life was natural, slow, difficult at times but healthy. In modern era, 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 knowledge of the specific plants to be used in specific disease and methods of application for particular aliments were passed down through oral tradition. 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) [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) [4]. 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) [5].Medicinal plants play a key role in human health care. 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. 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. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immune stimulatory, and antimicrobial properties have received research attention. *Sarpagandha* belongs to family Apocynaceae. It is native to the Indian subcontinent and East Asia [6][7]. *Sarpagandha* leaves extract is used to cure disease like cholera. *Sarpagandha* is known to contain phytochemicals like niacin, ascorbic acid, reserpine, riboflavin and serpentine [8]. 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.

A group of bacteria belonging to genus Vibrio generally cause cholera.*Vibrio cholerae* was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854 [9]. *V. cholerae* is a highly motile, comma shaped, halophilic, gram-negative rod [10]. Initial isolates are slightly curved, whereas they can appear as straight rods upon laboratory culturing. The bacterium has a flagellum at one cell pole as well as *pili*. The Vibrios tolerate alkaline media that kill most intestinal commensals, but they are sensitive to acid. *V.cholerae* is a facultative anaerobe, and can undergo respiratory and fermentative metabolism.They are comma shaped Gram negative bacteria.Vibrio habitatis a blackish or saltwater and attached themselves easily to the chitin containing shells of crabs, shrimps, and other shellfish [11].

Cholera is an infection of the small intestine by some strains of the bacterium *Vibrio cholerae* [12[13].Symptoms ranges from none, to mild, to serve [13]. The classic symptoms is large amounts of watery diarrhoea that lasts a few days. It is spread mostly by unsafe water and unsafe food that has been contaminated with human feces containing the bacteria [14]. Prevention methods against cholera include improved sanitation and access to clean water [15]. This study focuses on the identification of the phytochemical of *Rauvolfia serpentina* responsible to cure cholera caused by *Vibrio 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 *Sarpagandha* like niacin, ascorbic acid, reserpine, riboflavin and serpentine etc. It has already been established that *Rauvolfia sp* plant belonging to Apocynaceae 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 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 *Vibrio cholerae* bacteria. It has been found that isEnoyl-[acyl-carrier protein] reductase (protein database code 6IDE) is involved in Fatty acid biosynthesis metabolic pathway (BRENDA, KEGG) and very crucial for survival of the particular microbe [16][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 *Rauvolfia sp* plant were downloaded from the website [18]. The protein database code of theEnoyl-[acyl-carrier protein] reductase 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 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 theEnoyl-[acyl-carrier protein] 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. 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)[20].

Table 1 shows that Enoyl-[acyl-carrier protein] reductase enzyme–Niacin interaction has the highest positive value of -CDOCKER energy (18.5096) and minimum value of the difference (2.4776) between -CDOCKER interaction energy

and –CDOCKER energy followed by ascorbic acid. Thus the results indicated that niacin and ascorbic acid can effectively deactivate the with Enoyl-[acyl-carrier protein] reductase enzyme thereby interrupting the biological cycle of *Rouvolfia sp.* Higher positive values for niacin indicated that it was the most active ingredient against *Rouvolfia sp*. On the other hand, riboflavin can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Ajmaline and reserpine cannot interact with Enoyl-[acyl-carrier protein] reductase enzyme. Thus, the key phytochemicals preventing cholera caused by *Rouvolfia sp* is niacin.

CONCLUSION

It was previously known that *Rauvolfia serpentina* 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 (Niacin, Ascorbic acid, reserpine, Riboflavin and Ajmaline), which can have a significant interaction with the vital enzyme Enoyl-[acyl-carrier protein] reductase of the microbe. It was found that ascorbic acid and niacincan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Riboflavin is found to be not much effective in deactivating the enzyme of the microbe. Ajmaline and reserpine cannot deactivate the enzyme. Thus, this study could explain that the presence of ascorbic acid and niacin provided the medicinal values to *Rauvolfia serpentina* against cholera caused by *Vibrio cholerae*.

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Table 1. Results of Cdocking of Phytochemicals With Enoyl-[Acyl-Carrier Protein] Reductase (Receptor)

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN –CDOCKER INTERACTION ENERGY AND -CDOCKER ENERGY
1.	Niacin	18.5096	20.9872	2.4776
2.	Ascorbic acid	14.4456	30.682	16.2364
3.	Riboflavin	-10.9792	41.4079	52.3871
4.	Ajmaline	-181.419	-14.8613	196.2803
5.	Reserpine	Failed	Failed	NA

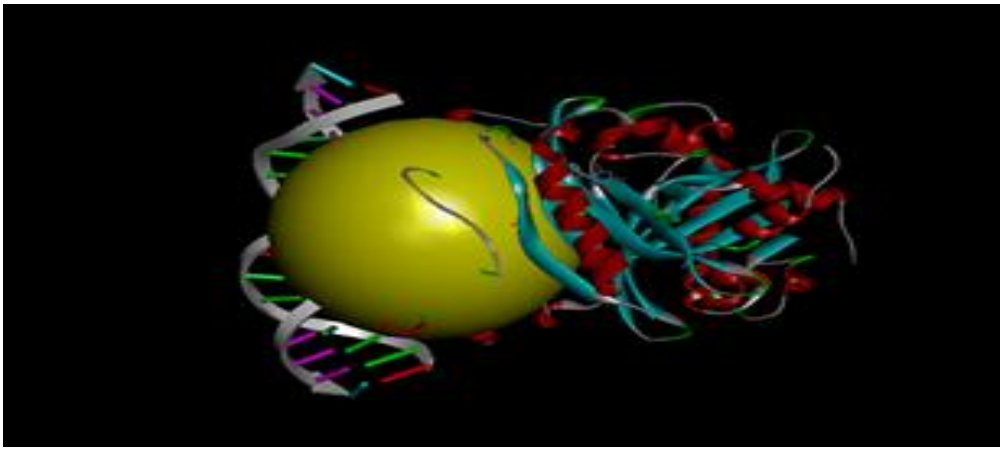

Fig. 1: Active Site of Enoyl-[Acyl-Carrier Protein] Reductase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Rauvolfia serpentina* against Alcohol Dehydrogenase of *Entamoeba histolytica* Causing Dysentery

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Rauvolfia serpentina* plant extract is used to cure dysentery. The plant extract contains different phytochemicals. Dysentery is caused 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 values for both the parameters indicated that out of different phytochemical thiamine and niacin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the life cycle of *Entamoeba histolytica*.

Key Words: Phytochemical, Biovia, Discovery studio, *Rauvolfia serpentina, Entamoeba histolytica*

INTRODUCTION

Dysentery and diarrhoea are major causes of morbidity and mortality in rural communities of developing world. In areas with poor sanitation nearly half of cases of diarrhoea and dysentery are due to *Entamoeba histolytica* [1]. *Entamoeba histolytica* affects millions of people and results in greater than 55,000 deaths a year [2]. It commonly occurs in less developed areas of Central and South America, Africa, and Asia [2]. Dysentery has been described at least since the time of Hippocrates [3]. The plant parts of Sarpagandha are used in treatment of dysentery. 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 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 [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].

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. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention.

Sarpagandha (*Rauvolfia serpentina*), the Indian snakeroot, devil pepper, or serpentine wood [7], is a species of the flowering plant in the family Apocynaceae [8]. An extract of Sarpagandha roots are used to cure disease like dysentery. *Rauvolfia serpentina* may cause adverse effects by interacting with various prescription drugs [9] or via interference with mechanisms of mental depression or peptic ulcer [9]. The reserpine in *R. serpentina* is associated with diverse adverse effects, including vomiting, diarrhoea, dizziness, headache, anxiety, or hypersensitivity reactions [8]. Sarpagandha known to contain phytochemicals like niacin, riboflavin, ajmaline, thiamine, ascorbic acid, reserpine [10]. There is high possibility that these phytochemicals play a major role in cure of dysentery. However, there is no report identifying the specific phytochemicals responsible to cure dysentery.

Entamoeba histolytica is an anaerobic parasitic amoebozoan, part of the genus *Entamoeba* [11]. Predominantly infecting humans and other primates causing amoebiasis. Transmission generally occurs by the ingestion of infected water or food due to fecal excretion of cysts, and even fecal-oral transmission within household and during male homosexual activity [12,13,14]. The reuse of human waste water has been identified as an important source of human infection. Infection can be asymptomatic or cause relatively mild intestinal upset and diarrhoea, or more severe amoebiasis in the form of amoebic characterized by stomach pain, bloody stools and fever. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.

Dysentery is an infection of the intestinal tract. Dysentery is a type of gastroenteritis that results in diarrhoea with blood [15,16] Other symptoms may include fever, abdominal pain, and a feeling of incomplete defecation [17,18,19]. Complications may include dehydration [20] The cause of dysentery is usually *Entamoeba histolytica* or *Shigella* [15]. Other causes may include certain chemicals, other bacteria, other protozoa, or parasitic worms [17]. Dysentery is managed by maintaining fluids using oral rehydration therapy. Efforts to prevent dysentery include hand washing and food safety measures while travelling in areas of high risk. Most vaccine development efforts are taking place in the public sector or as research programs within biotechnology companies. This study focuses on the identification of the phytochemical of *Rauvolfia serpentina* responsible to cure dysentery oral ulcer caused by *Entamoeba histolytica.*

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 *Rauvolfia serpentina* contains niacin, riboflavin, ajmaline, thiamine, ascorbic acid, and reserpine. It has already been established that *Rauvolfia serpentina* plant belonging to Apocynaceae 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 oral ulcer 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* bacteria. It has been found that alcohol dehydrogenase (protein database code 1Y9A) is involved in methionine metabolism, tyrosine metabolism, tryptophan metabolism, phenylalanine metabolism, leucine metabolism, valine metabolism, ethanol fermentation, propanol degradation (BRENDA) [21] and is very essential 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 *Rauvolfia serpentina* plant were downloaded from the website [22]. The protein database code of the alcohol dehydrogenase enzyme was identified from the website [23]. 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 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.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 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) [24].

Table 1 show that alcohol dehydrogenase-Thiamine interaction has the highest positive value of -CDOCKER energy (24.2471) and minimum value of the difference (0.0856) between - C DOCKER interaction energy and - C DOCKER

energy followed by niacin. Thus the results indicated that thiamine and niacin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *Rauvolfia serpentina.* Higher positive values for thiamine indicated that it was the most active ingredient against *Rauvolfia serpentina*. On the other hand, ajmalicine and reserpine can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing cholera caused by *Rauvolfia serpentina* are thiamine and niacin.

CONCLUSION

It was previously known that *Rauvolfia serpentina* plant has medicinal action against dysentery. Dysentery 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 (niacin, riboflavin, ajmalicine, thiamine, ascorbic acid, reserpine), which can have a significant interaction with the vital enzyme (alcohol dehydrogenase) of the microbe. It was found that thiamine and niacin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Ajmalicine, reserpine was found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of thiamine and niacin provided the medicinal values to *Rauvolfia serpentina* against dysentery 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	Ajmalicine	-12.2101	25.4462	37.6527
2	Ascorbic acid	1.8836	19.1093	17.2257
3	Reserpine	-4.40941	41.6	46.00941
4	Riboflavin	12.8024	31.6455	18.8431
5	Niacin	11.3484	13.5636	2.2152
6	Thiamine	24.2471	24.3327	0.0856

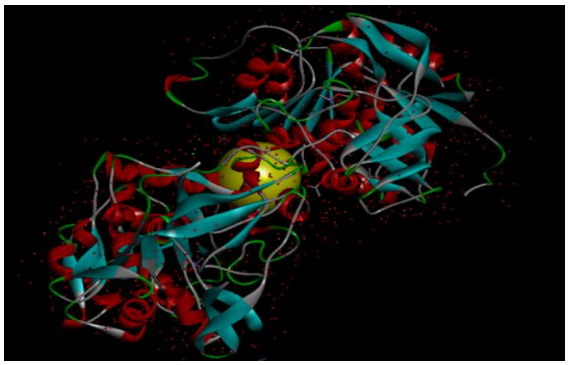

Fig. 1. Image of Receptor Ligand Interaction

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Tinospora cordifolia* against Laccase *Trichophyton rubrum* of Ringworm Causing Skin Disease

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ABSTRACT

Tinospora is considered as a bitter tonic and powerful immuno modulator. It is widely used herb as traditional medicines by indigenous groups. Phytochemicals analysis of medicinal plants attracts the attention of plant researchers due to development of more sophisticated tools. Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Tinospora cordifolia* plant extract is used to cure Skin disease. *Tinospora cordifolia* (Giloe/Guduchi) belonging to the family Menispermaceae**,** is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark. Plants based medicines, health products, pharmaceuticals, food supplements, cosmetics. The plant extract contains different phytochemicals. Skin disease is caused by Ring worm, *Microsporum*, *Streptococcus*, *Staphylococcus aureus*. One of the key enzymes involved in its biochemical pathway is Laccase-*Trichophyton rubrum.* It is a vital therapeutic plant used for the ethnomedical treatment of colds, headaches, pharyngitis, fever,diarrhea, oralulcer, diabetes, digestive disorders, skin disease. 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 Magnoflorine, Heptacosan-1-ol can effectively deactivate the enzyme thereby interrupting the life cycle of Ring worm.

Key Words: Phytochemical, BIOVIA, Discovery studio, *Tinospora cordifolia*, Ringworm.

INTRODUCTION

Tinospora cordifolia has been studied extensively for its adaptogenic activity. The whole and aqueous extracts are having significant adaptogenic activity on a variety of biological, physicals and chemical stressors on different model. In olden days, life is much harder but healthy and peaceful. 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.

Natural products have provided some of the important lifesaving drugs used of modern medicine. Natural products have been traditionally accepted as remedies for many diseases. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. Various medicinal properties are attributed to natural herbs. Medicinal plants represent the most source of recent prescription drugs and care product. 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). the scientific evaluation of *Tinospora cordifolia* for its medicinal efficacy which includes phytochemical screening, antimicrobial, antioxidant, and anticancer activities of the plant. 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). In modern medicine, *T. cordifolia* is used for the treatment of general weakness, fever, dyspepsia, dysentery, gonorrhoea, urinary diseases, viral hepatitis and anaemia. Plants play an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy. (DeviP.R., 2014). Higher plants as sources of medicinal agents for use in investigations prevention and treatment of diseases.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. Herbal medicines represent one of the most important fields of traditional medicine all over the world. 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., atropine from *Atropa belladonna* and morphine and codeine from Papaver *somniferum.* (Sahoo N.et al, 2010). Natural products with medicinal values are gradually gaining importance in clinical research. 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. A continuous and wide spread use of medicinal plants throughout the world has enhanced the concern over their safety, efficacy and quality of natural products. Thus, proper knowledge of the phytochemical constitutes of the plants is important, because this information will be desirable for synthesis of new products.

Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immunostimulatory, and antimicrobial properties have received research attention. Theever-increasing resistance of pathogens to antibiotics as well as the undesirable side effects of certain antimicrobial agents has necessitated the discovery of novel bioactive compounds. Antimicrobial activity of plant extracts was determined using Kirby-Bauer disc diffusion method. *Tinospora cordifolia* belongs to family Menispermaceae. *Tinospora* leaves extract is used to cure disease like Skin disease. *Tinospora* is known to contain phytochemicals like quercetin, Kaempferol, magnoflorine, palmatine, heptacosanol, Luteiolin, choline, sitosterol, berberine, columbin, syringin, phenol, nanocosan etc. There is high possibility that these phytochemicals play a major role in curing Skin disease. However, there is no report identifying the specific phytochemical responsible to cure skin disease.

A group of Laccase-*Trichophyton rubrum* belonging to the family Anthrodermataceae generally cause skin disease. Skin infection is common bacterial disease that affects the skin. Tinospora contains many different chemicals that might affect the skin. Some chemicals might have activity against cancer cells in test animals. Some chemicals have antioxidant effects. Other might increase the activity of body 's immune system. When giloy is taken with ghee on empty stomach in the morning, helps to cure all types of skin diseases including, acute & chronic dermatitis, prickly heat, sunburn, pruritus associated with urticaria, all forms of cosmetic allergy, ringworm, psoriasis, leucoderma and leprosy. This study focuses on the identification of the phytochemical of *Tinospora cordifolia* responsible to cure skin disease caused by Ring worm.

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 Tinospora cordifolia contains quercetin, Kaempferol, magnoflorine, palmatine, heptacosanol, Luteiolin, choline, sitosterol, berberine, columbin, syringin, phenol, nanocosanetc. It has already been established that *Tinospora* plant belonging to Menispermaceae family 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 Ring Worm

It has been reported that skin disease can cause as a result of Ring worm 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 Ring worm. It has been found that Laccase-Trichophyton rubrum (1KYA) is involved in helping of certain reaction but not involved in metabolic pathway(KEGG) and it helps in the formation of 4benzosemiquinone from 4benzediol 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. The protein database code of Laccase-*Trichophyton rubrum* 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 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 Laccase-*Trichophyton rubrum* 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 37.7691-2.08882 interaction has the highest positive value of -CDOCKER energy 35.68028 and minimum value of the difference 2.9005 between - C DOCKER interaction energy and - C DOCKER energy. Thus, the results indicated that 35.68028 can effectively deactivate the enzyme thereby interrupting the biological cycle of Ring worm. Higher positive values for indicated that it was the most active ingredient against Laccase-*Trichophyton rubrum* of Ring wormon the other hand, Palmatine, sitosterol can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing Skin disease caused by Ring worm are Magnoflorine Lutcolin, Kaempferol and nonacosane.

CONCLUSION

The therapeutic efficacy of *Tinospora cordifolia* makes it a primary drug in Indian Medicinal System. It was previously known that *Tinospora cordifolia* plant has medicinal action against skin disease. Skin disease is caused by Ring worm. This study was carried out to provide the theoretical basis of this observation. The plant is widely used in Ayurveda for treatment of several diseases like antispasmodic, anti-inflammatory, antiarthritic, anti-allergic and antidiabetic. Using Discovery studio module of BIOVIA software, molecular docking operation was performed to identify the phytochemical quercetin, Kaempferol, magnoflorine, palmatine, heptacosanol, Luteiolin, choline, sitosterol, berberine, columbin, syringin, phenol, nanocosan, which can have a significant interaction with the vital enzyme Laccase-*Trichophyton rubrum* of the microbe. It was found that Magnoflorine, Kaempferol, Lutcolin and Heptacosan-1-ol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Sitosterol, Palmatine were found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Magnoflorine, Lutcolin provided the medicinal values to *Tinospora cordifolia* against Skin disease caused by Ring worm. Phytochemical analysis of this plant may be useful in developing new specialized drugs with more efficiency. With so much to offer to the scientific world of medicine, the plant Tinospora truly acts as an incredible source.

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Table 1. Results of Cdocking of Phytochemicals With) Laccase-*Trichophyton Rubrum* (Receptor)

SL NO	LIGAND	-CDOCKER ENERGY	-CDOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN -C DOCKER INTERACTIONENERGY AND - C DOCKER ENERGY
1	Kaempferol	23.2967	28.4838	5.1871
2	magnoflorine	2.08882	37.7691	35.68028
3	palmatine	-4.86609	33.8347	38.70079
4	heptacosanol-1-ol	42.7065	51.3695	8.663
5	Luteiolin	27.6122	30.5127	2.9005
6	sitosterol	-33.1596	43.2646	76.4242
7	nanocosan	54.0153	53.4873	0.523

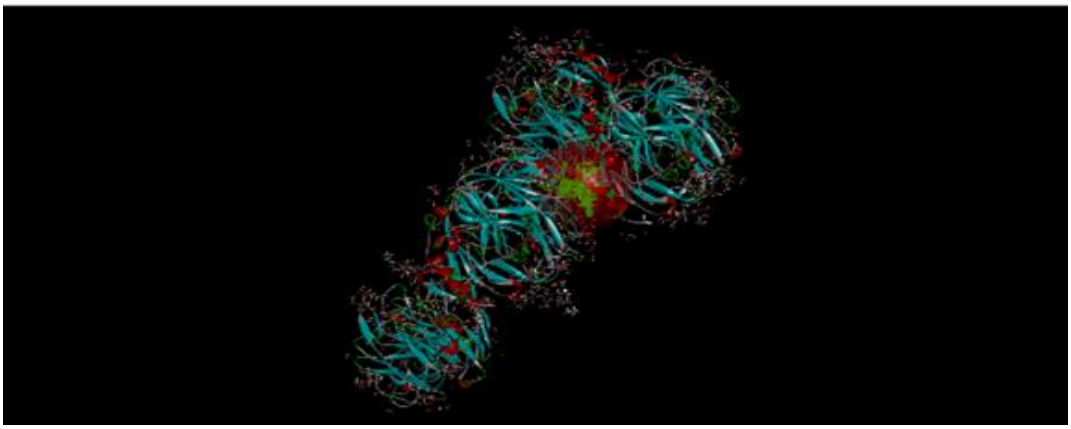

Figure 1. Active Site of Laccase-*Trichophyton rubrum* Enzyme.

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Tinospora cordifolia* against *Helicobacter pylori* Causing Ulcer

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Tinospora cordifolia* 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 microbial 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 Luteolin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the life cycle of *Helicobacter pylori*.

Key Words: Phytochemical, Biovia, Discovery studio, *Tinospora cordifolia*, *Helicobacter pylori.*

INTRODUCTION

In ancient days, life was very 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 can prevent our life from these dangerous hazards. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from different phytochemicals of plants. For thousands of years human has been using plant and their product for the treatment of numerous aliments. The traditional or the folk medicines comprises of knowledge and recently the internet in the study of medicinal plants as a source of pharmacologically active compounds has increased worldwide.

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. m [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. [2] Medicinal plants play a important role in human health care.

Giloya belongs to family menispermaceae. Giloya leaves extract is used to cure disease like Gonorrhea. This plant is known by the common names heart-leaved moonseed, gaduchi, and giloya. It is an herbaceous vine of the family Menispermaceae indigenous to the tropical areas of Bangladesh, India, Myanmar, and Sri Lanka.[3] Giloya is known to contain phytochemicals like Quercetin, Kaempferol, Magnoflorine, Palmatine, Heptacosanol, Luteolin, Choline, Sitosterol, Berberine, Columbin, Syringin, Phenol, Nanocosan, etc.[4] There is high possibility that these phytochemicals play a major role in curing ulcer. However, there is no report identifying the specific phytochemical responsible to cure ulcer.

A group of bacteria belonging to family Helicobacteraceae generally cause Ulcer. *Helicobacter pylori*, previously known as *Campylobacter pylori*, is a gram-negative, helically-shaped, microaerophilic bacterium usually found in the stomach. [5] Ulcer is a disease that primarily is a break in the inner lining of the stomach, the first part of the small intestine, or sometimes the lower esophagus. An ulcer in the stomach is called a gastric ulcer, while one in the first part of the intestines is a duodenal ulcer. The most common symptoms of a duodenal ulcer are waking at night with upper abdominal pain and upper abdominal pain that improves with eating. [6][7]. This study focuses on the identification of the phytochemical of *Tinospora cordifolia* responsible 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 *Tinospora cordifolia* contains quercetin, kempferol, magnoflorine, palmarine, heptacosano, luteolin, choline, sitosterol, berberine, columbin, syringin, phenol, nanocosan etc. It has already been established that *Tinospora cordifolia* plant belonging to Menispermaceae 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 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 alcohol dehydrogenase enzyme (protein database code 3TWO) 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*Tinospora cordifolia* plant were downloaded from the website [8]. The protein database code of the alcohol dehydrogenase 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 alcohol 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 [10]. Table 1 shows that Luteolin interaction has the highest positive value of -CDOCKER energy (31.5604) and minimum value of the difference (2.9551) between - C DOCKER interaction energy and - C DOCKER energy followed by kempferol. Thus the results indicated that Luteolin can effectively deactivate the alcohol dehydrogenase enzyme thereby interrupting the biological cycle of *Helicobacter pylori*. Higher positive values for Luteolin indicated that it was the most active ingredient against *Helicobacter pylori*. On the other hand, magnoflorine, palmarine, berberine and columbin can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Heptacosanol, sitosterol and nanocosan cannot interact with alcohol dehydrogenase enzyme. Thus, the key phytochemicals preventing ulcer caused by *Helicobacter pylori* are luteolin.

CONCLUSION

It was previously known that *Tinospora cordifolia* plant has medicinal action against ulcer. Ulcer 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 (quercetin, kempferol, magnoflorine, palmarine, heptacosano, luteolin, choline, sitosterol, berberine, columbin, syringin, phenol, nanocosan etc), which can have a significant interaction with the vital enzyme alcohol dehydrogenase of the microbe. It was found that luteolin can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. magnoflorine, palmarine, berberine and columbin, were found to be not much effective in deactivating the enzyme of the microbe. Heptacosanol, sitosterol and nanocosan cannot deactivate the enzyme. Thus, this study could explain that the presence of luteolin provided the medicinal values to *Tinospora cordifolia* 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	LUTEOLIN	31.5604	34.5155	2.9551
2	KEMPFEROL	27.5415	31.9382	4.3967
3	MAGNOFLORINE	-1.8178	34.7412	36.559
4	PALMARINE	-4.72023	32.6626	37.38283
5	BERBERINE	-16.9271	31.3175	48.2446
6	COLUMBIN	-41.0662	39.1365	80.2027
7	HEPTACOSANOL	FAILED	FAILED	FAILED
8	SITOSTEROL	FAILED	FAILED	FAILED
9	NANOCOSAN	FAILED	FAILED	FAILED

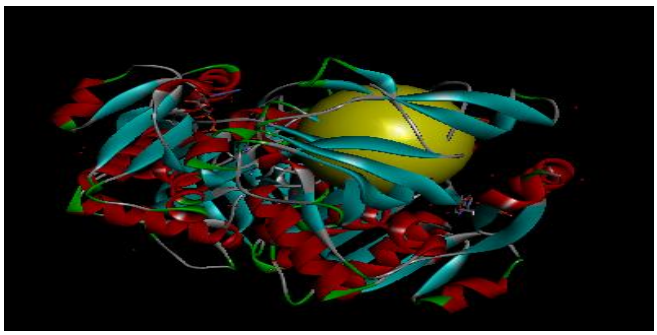

Figure 1. Active Site of Alcohol Dehydrogenase Enzyme

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RESEARCH ARTICLE

In silico Analysis of Phytochemicals from *Vaccinium corymbosum* (Blue berry) against Sore Throat

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ABSTRACT

Phytochemicals are non-nutritive compounds obtained from plants. It has been reported that *Vaccinium corymbosum* plant 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 Skikimate 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 Caffeic Acid, Aloe-emodin, Anthraquinone, Apigenin, Resveratrol and Cinnamyl-Alcoholcan effectively deactivate Skikimate dehydrogenase enzyme. Thereby interrupting the life cycle of *Haemophilus influenzae.*

Key Words: Phytochemical, biovia discovery studio, *Vaccinium corymbosum, Haemophilus influenzae.*

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. Mostly people in city are very busy in day to day life and have a bad food habits due to which they face many diseases. Diseases like acidity, diabetes, ulcer, blood pressure etc. To cure these diseases peoples take different medicines but without consulting any doctor. As aresult it may cause side effects and gives resistance to that microbe. 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). Some 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 (G. Schmeda-Hirschmann and A. R. De Arias, 1992). 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 numerous medicinal properties like anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes actions. (Medini F et. al, 2015). Apart from these all the medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities in our country and also in foreign.

Plants have been used for medicinal purposes long before prehistoric period and it plays key role in human health care. 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 medicine like allopathic medicine, herbal medicine, alternative medicine, homeopathy, and aroma therapy (SekharM et. al 2007). Medicinal plants include various type of plants used in herbalism. It is use of plants for medicinal purposes, and the study of such uses. Prescribed today on modern medicinal system about 25% of modern pharmaceutical drugs have botanical origin. The breast cancer fighting drug taxol comes from the specicyew tree, quinidine from *Cinchona spp*, vincristrine and vinblastine from *Catharanthous roseus*. Atropine from atropa belladonna, codeine and morphine form *papaver somniferum.* Recently, WHO estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have medicinal characteristics. 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 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 plant. Plant that demonstrated anti-cancer, anti-oxidant,anti-inflammatory immunostimulatory and anti-microbial properties has received research attention.*Vaccinium corymbosum* belongs to family Ericaceae. *Vaccinium corymbosum* seeds extract in used to cure disease like Sore throat. *Vaccinium corymbosum* is known to contain phytochemicals like Anthraquinone, Apigenin, Resveratrol, Aloe-emodin, Cercumin Synthetase, Caffeic Acid, Cinnamyl-Alcohol (Bart N, 2011). There is high possibility that these phytochemicals play a major role in curing Sore throat. Sorethroat is cause by the bacteria known asHaemophilus influenzae, gram negative *coccobacillus* bacteria. *Haemophilus*bacteria are highly contagious. They can spread through airborne droplets i.e. by contamination, or through shared food or drinks.Sorethroat occurs most commonly in children. Although sore throat can occur anytime but it tends to circulate in winter and early spring very fastly. Sorebacteria flourish wherever groups of people are in close contact.

Sore throat can lead to serious complications. Antibiotic treatment reduces the risk. Serum antibody to the capsule or to somatic antigens is bactericidal and promotes phagocytosis. This study focuses on the identification of the phytochemicals of *Vaccinium corymbosum* responsible to cure Sore throat caused by *Haemophilus influenzae.* Different extracts of *Vaccinium corymbosum* plant are help to block the metabolic pathways of the microbe as a result growth of microbe is stop and it may lades to the death of that microbe.

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

Enzyme Found in *Haemophilus influenzae*

It has been reported thatcan cause as a result of *Haemophilus* 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 *Haemophilus spp.* Bacteria. It has been found that Skikimate dehydrogenase enzyme (protein data base 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 microbial protein to successfully inhibit the microbe.The discovery studio module ofbiovia software was used for identifying molecular interaction and perform molecular docking. In this process, first the sdf files for the phytochemicals found in the *Vaccinium corymbosum* plant were downloaded from the website (www.molinstinct.in). The protein data base code of the beta lactamase enzymewas identified from the website (www.brendaenzymes.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 Skikimate 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 (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 Skikimate dehydrogenase -Caffeic Acidinteraction has the highest positive value of -CDOCKER energy (31.2404) and minimum value of the difference (1.02) between - C DOCKER interaction energy and - C DOCKER energy followed by Aloe-emodin, Anthraquinone, Apigenin, Resveratrol and Cinnamyl-Alcohol. Thus the results indicated that Caffeic Acid and Aloe-emodin can effectively deactivate the Skikimate dehydrogenase enzyme thereby interrupting the biological cycle of *Haemophilus sp.* Higher positive values for Caffeic Acidindicated that it was the most active ingredient against *Haemophilus sp.* On the other hand, Cercumin Synthetase can deactivate the enzyme to a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing diarrhea caused by *Haemophilus sp.* are Caffeic Acid, Aloe-emodin, Anthraquinone, Apigenin, Resveratrol and Cinnamyl-Alcohol.

CONCLUSION

It was previously known that *Vaccinium corymbosum* plant has medicinal action against diarrhea. Diarrhea is caused by *Haemophilus 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 (Anthraquinone, Apigenin, Resveratrol, Aloe-emodin, Cercumin Synthetase, Caffeic Acid, Cinnamyl-Alcohol), which can have a significant interaction with the vital enzyme (Skikimate dehydrogenase) of the microbe. It was found that Caffeic Acid, Aloe-emodin, Anthraquinone, Apigenin, Resveratrol, Cinnamyl-Alcohol can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Cercumin Synthetasewere found to be not much effective in deactivating the enzyme of the microbe. Thus, this study could explain that the presence of Caffeic Acid, Aloe-emodin, Anthraquinone, Apigenin, Resveratrol, Cinnamyl-Alcoholprovided the medicinal values to *Vaccinium corymbosum* against diarrhoea caused by *Haemophilus Sp.*

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Table 1: Results of CDocking of Phytochemicals with Skikimate Dehydrogenase (Receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	DIFFERENCE BETWEEN- C DOCKER INTERACTION ENERGY AND - C DOCKER ENERGY
1	Caffeic Acid	30.2204	31.2404	1.02
2	Aloe-emodin	23.4109	28.0769	4.666
3	Anthraquinone	8.07161	15.7765	7.70489
4	Apigenin	16.72878	25.2499	8.52112
5	Resveratrol	19.3412	29.4724	10.1312
6	Cinnamyl-Alcohol	5.74496	19.4129	13.66794
7	Cercumin Synthetase	-3.26194	24.34371	27.60565

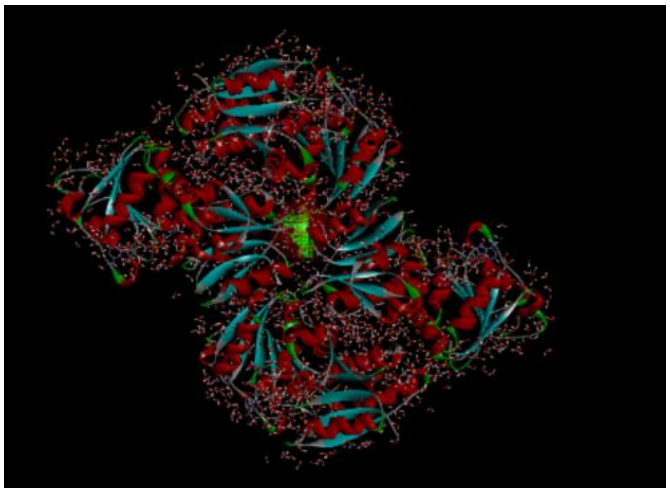

Figure 1. Active Site of Skikimate Dehydrogenase Enzyme

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RESEARCH ARTICLE

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

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ABSTRACT

Phytochemicals are compounds that are produced by plants. It has been reported that *Alpina galangal* plant extract is used to cure bronchitis. The plant extract contains different phytochemicals. Bronchitis is caused by *Streptococcus pneumoniae.* One of the key enzymes involved in its biochemical pathway isadenylate kinase. Bronchitis is an infection of the main airways of the lungs causing them to become irritated and inflamed. 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 1′acetoxychavicol acetate, 1′s-1′-acetoxyeuginol acetate and p-hydroxycinnamoaldehyde can effectively deactivate the adenylate kinase enzyme thereby interrupting the life cycle of the microbe.

Keywords: phytochemical, Biovia, Discovery studio, *Alpina galangal, Streptococcus pneumoniae*

INTRODUCTION

Life was natural, slow, difficult at times but healthy during olden times. Today, in modern times, lifestyle has become 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. So now it's a high time for all of us to be cautious towards our health. Nature has been a source of medicinal agent 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. (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 breast-cancer-fighting drug Taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristine and vinblastine from *Cataractous roseus,* atropine from Atropos belladonna and morphine and codeine from *Papaver somniferous*. (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 whichhave received research attention demonstrated anticancer, antioxidant, anti-inflammatory, immune-stimulatory, and antimicrobial properties. It is not always possible to prevent acute or chronic bronchitis, but several things can reduce the risk.

These include

avoiding or quit smoking

- avoiding lung irritants, such as smoke, dust, fumes, vapors, and air pollution
- wearing a mask to cover the nose and mouth when pollution levels are high
- washing the hands often to limit exposure to germs and bacteria
- asking about vaccinations to protect from pneumonia and the flu.

Galangal belongs to family zingiberaceae. Galangal leaves extract is used to cure disease like diarrhoea (reference). Galangal is known to contain phytochemicals like beta pinene, beta farnesene, 1-acetoxychavicol acetate, beta bergamotene, beta bisabolene, alpha fenchyl acetate, etc. There is high possibility that these phytochemicals play a major role in curing bronchitis. People with bronchitis have swelling and inflammation in their bronchial tubes, the air passages that link the mouth and nose with the lungs. Symptoms of bronchitis include a cough, wheezing, and difficulty breathing. People may also have trouble clearing heavy mucus or phlegm from their airways. Bronchitis can be acute or chronic. Acute bronchitis usually clears up, but chronic bronchitis is persistent and never completely goes away. Quitting or avoiding smoking can help prevent bronchitis. This study focuses on the identification of the phytochemical of *Alpina galangal* responsible to cure bronchitis caused by *Streptococcus pneumoniae.*

MATERIALS AND METHODS

Software used

Discovery studio module of Biovia software (Dassault Systemsof 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 *Alpina galangal* contains beta pinene, beta farnesene, 1-acetoxychavicol acetate, beta bergamotene, beta bisabolene, alpha fenchyl acetate, etc.It has already been established that *Alpina galangal* plant belonging to Apiaceae 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

It has been reported that bronchitis can causeas a result of *Streptococcus pneumoniae* 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 adenylate kinase enzyme (protein database code 3S46) is involved in alanine and aspartate and D-alanine 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 *Alpina galangal* plant were downloaded from the website (pubchem). The protein database code of the adenylate kinase enzyme was identified from the website (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 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 the adenylate kinase 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 1’acetoxy chavicol acetate interaction has the highest positive value of -CDOCKER energy (30.6158) and minimumvalue of the difference (5.6663) between - C DOCKER interaction energy and - C DOCKER energy followed by 1’s-1’-acetoxyeuginol acetate. Thus, the results indicated that 1’acetoxy chavicoland 1’s-1’-acetoxyeuginol acetatecan effectively deactivate the enzyme thereby interrupting the biological cycle of *Streptococcus pneumoniae*.

Higher positive values indicated that it was the most active ingredient against *Streptococcus pneumoniae*. On the other hand, beta pinenecan deactivate the enzymeto a small extent (negative -CDocker energy but positive -CDocker interaction energy). Thus, the key phytochemicals preventing bronchitis caused by *Streptococcus pneumoniae* are1′acetoxy chavicol acetate,1′s-1′-acetoxyeuginol acetate, p-hydroxy cinnamaldehyde, beta pinene.

CONCLUSION

It was previously known that *Alpina galangal* plant has medicinal action against bronchitis. Bronchitis 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 (beta pinene, beta farnesene, 1-acetoxychavicol acetate, beta bergamotene, beta bisabolene, alpha fenchyl acetate, etc.), which can have a significant interaction with the vital enzyme (adenylate kinase) of the microbe.It was found that1′acetoxychavicol acetate, 1′s-1′-acetoxyeuginol acetate, p-hydroxy cinnamaldehydecan form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Beta bergamotene and alpha fenchyl acetate were found to be not much effective in deactivating the enzyme of the microbe. Beta sisterol arabinoside, beta bisabolene cannot deactivate the enzyme. Thus, this study could explain that the presence of these phytochemical provided the medicinal values to *Alpina galangal* against bronchitis caused by*Streptococcus pneumoniae*.

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Table 1. Results of C-Docking of phytochemicals with adenylate kinase (receptor)

SL NO	LIGAND	- C DOCKER ENERGY	- C DOCKER INTERACTION ENERGY	Difference between- C DOCKER interaction energy and - C DOCKER energy
1	1′acetoxy chavicol acetate	24.9495	30.6158	5.6663
2	1′s-1′-acetoxyeuginol cetate	15.8578	27.5764	11.7186
3	p-hydroxycinnamaldehyde	17.2029	21.0432	3.8403
4	beta pinene	-6.6876	13.7605	20.4481
5	Beta farnesene	-26.587	27.0236	53.6106
6	Beta bergamotene	-23.2303	24.317	47.5473

7	Beta sistosterolarabinoside	-38.9411	37.9951	76.9362
8	Beta bisabolene	-36.6439	26.4692	63.1131
9	Alpha fenchyl acetate	-20.7865	23.1112	43.8977

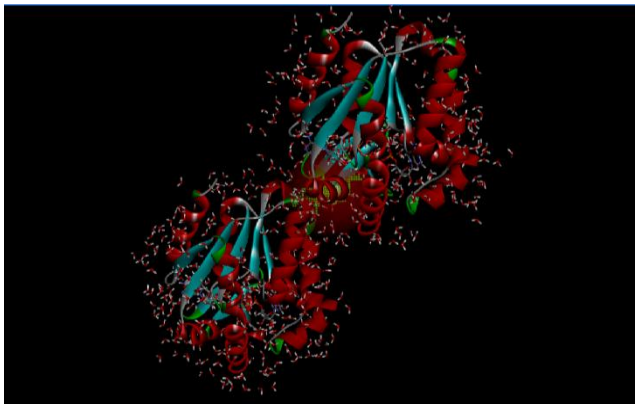

Figure 1. Active site of adenylate kinase enzyme