Indian Journal Of Natural Sciences *ISSN: 0976 - 0997*

Vol 1 / Issue 3 / December 2010 *© IJONS*

RESEARCH ARTICLE

Occurrence of House Sparrow *Passer domesticus indicus* L. in Sivakasi, Virudhunagar District, Tamil Nadu, India.

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Received: 15 Oct 2010 Revised: 10 Nov 2010 Accepted: 28 Nov 2010

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ABSTRACT

In India there are reports that house sparrow populations are under threat. The survey was carried out in market and residential areas in Sivakasi Taluk, Virudhunagar District, Tamil Nadu, India. The study revealed that the mean number of house sparrow in residential areas ranged from 9 individual to 18 individuals. Kanthapuram colony showed highest number of house sparrows. The mean number of house sparrows in market areas ranged from 8.6 to 12.8. The vegetable shop area and Parasakthi colony supported high number of sparrows. Only one nesting site was observed in each study site of market area. In residential area the highest number i.e. 3 nesting sites were observed in Kanthapuram colony. House sparrow number over the last decade has fallen. The decline in bird population over the year has been inferred by survey conducted locally. Many theories put forward to explain such as a decline include pollution, increase in predators and loss of a reliable food source. The disappearance of house sparrow seems to be connected to the way we build our houses and the ease with birds can find food. Many new house designs and home improvements have restricted the number of suitable nooks and crannies for the house sparrows to nest in.

Key words: House sparrow- *Passer domesticus indicus* L, decline, population, food sources, home improvements, residential and market areas

INTRODUCTION

Among the various species of birds, the house sparrow *Passer domesticus indicus* L. is one of the familiar species that has followed man everywhere and is inseparable from human habitations. The non-migratory sparrows are widely distributed in the Indian subcontinent and occur worldwide [1]. The house sparrow is one of the most common bird in the urban environment worldwide, and is ecologically tightly linked to this

special habitat. It occupies a variety of environments along the urban gradient [2]. House sparrows are well known for their frequent cheeping and chirruping from roof and gutters [3]. Although the effects of urbanization on avian community composition are well known, there is much less information about how individual birds are affected by these human-generated habitat differences [4]. The reasons for the declining of house sparrow population includes loss of invertebrates, reduction in winter seed food, change in agricultural practices, loss of grass and lawns from gardens, loss of nest sites, predation, pesticide use, changing architecture of human habitation, lack of traditional granaries[3]. In India also there are reports that house sparrow populations are under threat. Hence, the present study "Occurrence of House Sparrow *Passer domesticus indicus* L. (PASSERIFORMES: PLOCEIDAE) in Sivakasi" was undertaken

METHOD OF STUDY

Study Area

The study was undertaken in Sivakasi, a town in Virudhunagar District of Tamil Nadu, India. The survey was carried out from December 2008 to March 2009. In Sivakasi 14 places were selected for survey. They are categorized into market area and residential area.

Method - Walkover Survey

The number of House Sparrow was counted by following walkover survey method. The house sparrows are active from 8.00 a.m. to 11.00 a.m. In each study site, the number of birds was counted by 30 minutes observation. The nesting sites of house sparrow were also observed.

RESULTS AND DISCUSSION

The study revealed that the house sparrow population was higher in residential area. The house sparrow is an omnivorous bird. It feeds on grains, fruits, insect and insect larva. The house sparrow is present only where the man inhabited. Mostly the house sparrows build their nests in residential area. Among the residential area more number of nests was observed in Kanthapuram colony. This is due to availability of nesting sites. In Kanthapuram colony lot of old buildings are available. It facilitates the house sparrow to build the nest. The high number of house sparrows in Kanthapuram colony may also be due to the availability of food sources which support a good population of insects (Table 1, 3). Rajashekar [1] reported that in Bangalore the house sparrows built their nest on crevices of hatched roofs of old houses, electric pipelines and ventilation holes.

The lowest number of house sparrow was observed in residential area found along the N.R.K.R. Street. The mean number is 9.0. This road is always busy with heavy traffic and human activity. This may be the reason for the less population. Leveau [5] reported that in Argentina significantly lower house sparrow population was observed during weekend days when the pedestrian and vehicle traffic increased when compared to working days. Car traffic noise appears to diminish habitat quality, due to an increase of the stress and cause distortion in vocal communication. On the other hand, vehicle movement also can disturb the house sparrow foraging activity. In market area the house sparrow population was high in vegetable shop area. The mean number is 12.8. The food sources are high in that area but the availability of nesting site is poor. Only one nest has been identified in that area (Table 2). In Bangalore also market areas supported only a moderate number of house sparrows [1]. The house sparrow number declined in Luv mainly due to changes in urban habitats resulting from urbanization process such as, contracting of the residential areas, development of new micro-habitats with very little greenery and architecture unsuitable for nest construction building houses in rural areas covered with weeds [6]. To conserve the house sparrow

future conservation plans and status assessment, either at the national or international level should be carried out for house sparrows these taxa should be regarded as designable units [7].Many reasons are given for disappearance of house sparrows. The introduction of unleaded petrol is one, as the combustion of which produces compounds methyl nitrite, which is highly toxic for small insects that forms a part of a sparrow chick's diet. As supermarkets mushroom in urban areas, the old fashioned grain shops are disappearing. It was once common sight to see flocks of sparrows feasting on the grain in the gunny sacks displayed in front of these shops or on the spilt grain. Urbanization has done away with home gardens, which had worms and insects for the young sparrows. But pesticides have proved lethal for their survival. The most recent reason for their disappearance is the mobile phone towers. The waves from the tower are capable of destroying the life in the eggs. Thereby they are incapable of hatching. Reduction of nest-site availability due to improvements in quality and insulation of rooftops and a reduction of food availability due to loss of weedy corners from urban areas have both been suggested as likely explanations, as was in increased transmission of epidemic diseases.

ACKNOWLEDGEMENT

The authors express the profound thanks to the Management and Head of the Department of Zoology. Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi for providing facilities to carry out this work.

Table 1: The number of House Sparrow in Residential Area of Sivakasi

S.No.	Place	Number of House Sparrow					$\bar{x}$
1	Ammankoil Patti	13.12.08	04.01.09	14.02.09	14.03.09	29.03.09	
		11	9	10	10	13	10.6
2	N.R.K.R. Street	13.12.08	15.01.09	15.02.09	15.03.09	29.03.09	
		6	8	10	12	9	9.0
3	Muslim Street	14.12.08	15.01.09	15.02.09	15.03.09	29.03.09	
		16	12	17	13	15	14.6
4	Pudhu Colony	21.12.08	16.01.09	22.02.09	15.03.09	29.03.09	
		8	12	10	9	12	10.2
5	Kamarajar Puram Colony	25.12.08	17.01.09	01.03.09	16.03.09	05.04.09	
		15	16	17	13	14	15.0
6	Kanthapuram Colony	25.12.08	17.01.09	08.03.09	16.03.09	05.04.09	
		17	20	18	19	16	18.0
7	Vivekanandhar Colony	28.12.08	18.01.09	08.03.09	16.03.09	05.04.09	
		15	14	15	12	13	13.8
8	Bose Colony	28.12.08	18.01.09	08.03.09	16.03.09	05.04.09	
		12	15	13	11	12	12.6
9	Subramania Puram Colony	29.12.08	25.01.09	10.03.09	22.03.09	07.04.09	
		12	18	17	15	14	15.2
10	Kanna Nagar	29.12.08	25.01.09	10.03.09	22.03.09	07.04.09	
		10	7	10	13	12	10.4

Table 2: The number of House Sparrow in Market Area of Sivakasi

S.No.	Place	Number of House Sparrow					x̄
1	Near Sivan Kovil	09.12.08	04.01.09	14.02.09	14.03.09	28.03.09	
		7	10	12	8	9	9.2
2	Vegetable shop area	21.12.08	16.01.09	25.02.09	15.03.09	28.03.09	
		15	10	18	11	10	12.8
3	Mani Nagar	01.01.09	26.01.09	14.03.09	22.03.09	28.03.09	
		8	5	7	12	11	8.6
4	Parasakthi colony	01.01.09	26.01.09	14.03.09	22.03.09	28.03.09	
		12	17	10	13	12	12.8

Table 3: The number of nesting sites of House Sparrow in Sivakasi

S.No.	Place	Number
	Market Area	
1.	Near Sivan Koil	1
2.	Vegetable shop area	1
3.	Mani Nagar	1
4.	Parasakthi Colony	1
	Residential Area	
5.	Ammankoil Patti	2
6.	N.R.K.R. Street	1
7.	Muslim Street	2
8.	Pudhu Colony	1
9.	Kamarajar Puram Colony	1
10.	Kanthapuram Colony	3
11.	Vivekananthar Colony	1
12.	Subramaniapuram Colony	2
13.	Bose Colony	1
14.	Kanna Nagar	1

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Indian Journal Of Natural Sciences *ISSN: 0976 - 0997*

Vol 1 / Issue 3 / December 2010 *© IJONS*

RESEARCH ARTICLE

Phytochemical and Anti Bacterial Activity of *Tridax procumbens* L.

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Received: 16 Aug 2010 Revised: 25 Nov 2010 Accepted: 1 Dec 2010

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ABSTRACT

The whole plant of *Tridax procumbens* L. family Asteraceae were extracted with three different solvents i.e, pet-ether, aqueous and ethanol. The crude extract fractions pet-ether, aqueous and ethanol of the plant *Tridax procumbens* L. found to contain Alkaloids, Steroids, Protein and Amino acids. *Tridax procumbens* L. investigated for its preliminary phytochemical and anti bacterial activity. The extracts showed significant anti bacterial activity against all the micro organisms tested and the effect so produced was compared with standard drug Gentamycin. However pet-ether shows the moderate anti bacterial activity against the entire micro organism.

KEY WORDS; Phytochemical, Anti bacterial, *Tridax procumbens* L., Gentamycin

INTRODUCTION

Tridax procumbens L. (Asteraceae) is a weed found abundantly in agriculture fields and waste lands. It grows to about 45 cm tall and it has attractive flowers and leaves. It occurs in southern India states like Tamilnadu, Andra Pradesh, Kerala and Karnataka. Extensive literature survey reveals that studies on Hypoglycemic activity [1], Hemostatic activity [2], Anti oxidant activity [3], Hepatoprotective effect [4], Wound healing activity [5], Hypotensive activity [6] and Hepato toxicity[7], GC-MS analysis [8] of volatile compounds of *Tridax procumbens* L. resulted in the identification of sterols like Camphosterol, Stigmasterol, Beta-Ceto sterol. It is used by the local peoples to control Haemorrhage in wounds. In the present study, different fractions of the extracts of the plant have been investigated for Anti bacterial activity of *Tridax procumbens L.*

MATERIALS AND METHODS

Plant material

The *Tridax procumbens* L. were collected from the lands of Anaikatti area in Coimbatore District of Tamil Nadu by uprooting the whole plant. It was identified by G.V.S.Moorthy, Joint director, Botanical survey of India, Tamil Nadu Agricultural University Campus (TNAU) Coimbatore. (Specimen no.BSI/SC/5/23/09-10/Tech-1143). All parts of the plant were removed carefully and shaken to remove unwanted particles like sand and soil. Drying was done under the shade. Shade drying was preferred to avoid denaturation of phytochemical constituents.

Preparation of extracts

Dried plant were reduced in size to coarse powder with the aid of suitable means and three portions, each 150 gm was reserved for extraction with three different solvents like Petroleum Ether, Ethanol and aqueous extract. Size reduction provides more contact area for solvent penetration and facilities effective for extraction of phytochemicals. The powdered plant material was subjected to extraction in a soxhlet apparatus for 72 hours each using pet-ether (60-80^{0}C), ethanol and aqueous respectively [9].

Preliminary phytochemical studies

All the fractions were subjected to preliminary phytochemical investigation for the presence of secondary metabolites such as alkaloids, carbohydrates, proteins and amino acids, saponins and flavonoids utilizing standard methods of analysis.

Anti bacterial screening [10]

Anti bacterial activity of the plant was conducted by using Disc diffusion method. Nutrient agar media constituents were mixed with sufficient quantity of distilled water. Transfer into a boiling tube a quantity of 30ml. The tubes were autoclaved for 15min at a pressure of 15 ps/sq inch pressure. The selected micro organisms were *Staphylococcus aureus, Micrococcus luteus, Proteus mirabilis* and *Shigella boydii* . They were inoculated in the boiling tube. Later this was transferred into Petri dishes and kept on a flat surface. The test solution and standard solution of the concentration 1200μg/ml and control solution were taken in the Petri dishes and marked. The disc of similar size were made and soaked in the standard (Gentamycin),test and control solution and placed in the petri dishes at a distance. The Petri dishes were incubated at 37^{0}C for 24 hours. Inhibition of microbial growth was determined by absorbing the zone of inhibition both in test and standard.

Thin layer chromatography [11]

Thin layer chromatography of pet-ether, ethanol and aqueous extract of *Tridax procumbens L* was carried out in different solvent system and best resolving system was choosen for running the plates. The plates were then exposed to various detecting systems.

RESULTS

The pet-ether and ethanol extract revealed the presence of alkaloids, carbohydrates, steroids, proteins and amino acids, gums and mucilages while the aqueous extract has shown the presence of alkaloids, steroids, tannins, phenolic compounds, saponins and flavonoids. The results were shown in table 1. Thin layer

chromatographic analysis of crude extract prevailed the presence of steroids, carbohydrates and alkaloids. The results of TLC analysis of various extract of *Tridax procumbens* were shown in Table 2. Zone of inhibition was clearly observed in the Petri dishes cultured with test micro organisms both in test and standard. The pet-ether extract shows moderate anti bacterial activity. The observed zone of inhibition were 16,12,10,7 mm in the test sample of pet-ether extract for organisms *Staphylococcus aureus, Micrococcus luteus, Proteus mirabilis, Shigella boydii* respectively. Similarly ethanol extract showed the zone of inhibition 7,7,5,3 mm for *Staphylococcus aureus, Micrococcus luteus, Proteus mirabilis and Shigella boydii* respectively. Similarly Aqueous plant extract showed zone of inhibition 3,3,2,0mm for *Staphylococcus aureus, Micrococcus luteus, Proteus mirabilis* and *Shigella boydii* respectively. The reports were shown in Table 3.

CONCLUSION

Various extract of *Tridax procumbens* were subjected to preliminary phytochemical investigation and it showed the presence of alkaloids, carbohydrates, proteins and amino acids, saponins and flavonoids. Thin layer chromatographic analysis of crude extract confirmed the presence of steroids, carbohydrates and alkaloids. The anti microbial activity were evaluated by using three different extract of *Tridax procumbens* against selected *Staphylococcus aureus, Micrococcus luteus, Proteus mirabilis and Shigella boydii* organism. Gentamycin was used as a standard drug. Among the three extract pet-ether showed the moderate anti bacterial activity on above organisms. Ethanol extract showed less activity compared to pet-ether extract. Aqueous extract did not show any activity for *Shigella boydii.* Thus our investigation reveals that the plant *Tridax Procumbens L* has anti bacterial substances.

Table 1: Data Showing the Qualitative Phyto Chemical Analysis of *Tridax procumbens* L.

S. No.	Name of the phyto constituents	Petroleum ether extract	Ethanol extract	Aqueous extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	-
3	Glycosides	-	-	-
4	Steroids	+	+	+
5	Fixed oils & Fats	-	-	-
6	Triterpenoids	-	-	-
7	Proteins and Amino acids	+	+	-
8	Tannins Phenolic Compounds	-	-	+
9	Saponins	-	-	+
10	Gums and Mucilage	+	+	-
11	Flavones and Flavonoids	-	-	+

(+) Present; (-) Absent.

Table 2: TLC of petroleum ether, ethanolic extract & aqueous extract of *Tridax procumbens* L.

		Colour of spots observed				R_f Value		
			UV					
S.No	Extracts	Day light	Short UV	Long UV	Iodine chamber	Spot 1	Spot 2	Spot 3
1	Petroleum ether	Green	Green	Brown	Brown	0.98	0.97	0.96
2	Ethanol	Green	Green	Brown	Brown	0.98	0.96	0.97
3	Aqueous	Green	Green	brown	Brown	0.97	0.96	0.98

Table 3: Anti bacterial activity of petroleum ether, ethanolic extract & aqueous extract of *Tridax procumbens* L.

		Zone of Inhibition(mm)					
S.No	Organism	Petroleum ether		Ethanol		Aqueous	
		Test	Std	Test	Std	Test	Std
1	*Staphylococcus aureus*	16	20	7	31	3	23
2	*Micrococcus luteus*	12	28	7	15	3	19
3	*Proteus mirabilis*	10	20	5	20	2	32
4	*Shigella boydii*	7	23	3	10	0	0

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Indian Journal Of Natural Sciences *ISSN: 0976 - 0997*
Vol 1 / Issue 3 / December 2010 *© IJONS*

RESEARCH ARTICLE

Analysis of Hydrophobic Activity of Drugs for Diabetes Mellitus

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Received: 10 Oct 2010 Revised: 25 Nov 2010 Accepted: 2 Dec 2010

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ABSTRACT

Nowadays, Diabetes is a challengeable disease in India. A large work has been focussed on analysis of hydrophobic activity of drugs for diabetes mellitus. In this work we address a specific suitable ligand for diabetes mellitus. We have been retrieved that the Gene and Protein which is responsible for diabetes mellitus and target binding site has been identified. The list of drugs is retrieved from drug bank which are used to treat diabetes mellitus and the best ligand has been identified based on molecular docking. We are highly concentrated in the hydrophobic activity of the ligand. Finally we have observed that the ligand acetohexamide has the highest hydrophobic activity when compared with other ligands. Hydrophobic activity is the most essential parameter in all the drug molecules.

Keywords: Diabetes mellitus, Drug designing, Ligands, hydrophobic activity, Docking

INTRODUCTION

The risk of diabetes is markedly higher (two- to three-fold) in patients with schizophrenia compared with the general population and evidence suggests a similar increased incidence of diabetes in patients with bipolar disorder and schizoaffective disorder The main potential metabolic concerns in addition to the risk of developing type II diabetes are the risk for cardiovascular disease (CVD) and shorter life-expectancy. Mortality among patients with schizophrenia is higher than among the general population, and CVD accounts for a significant proportion of this excess mortality. [1]

Diabetes mellitus

Type 1 diabetes is an autoimmune disease. An autoimmune disease results when the body's system for fighting infection turns against a part of the body .Type 2 diabetes is most often associated with older age, obesity, physical activity and certain ethnicities. In Pre-diabetes, blood glucose levels are higher than normal but not high enough to be characterized as diabetes. Pre-diabetes also increases the risk of heart disease and stroke with weight loss and physical activity. Diabetes mellitus is a complex, multifactor and polygenic disease likely to be caused by one or more gene alterations action in combination with non-genetic factors.

Insulin is a hypoglycemic hormone and it is composed of two peptide chains referred to as chain A and chain B. These chains are linked together by two disulfide bonds. Insulin is a small protein with a molecular weight of about 6000 Daltons. It is synthesized in significant quantities only in beta cells of the Pancreas. [2]

Hydrophobic activity

In contrast to many traditional pharmaceutical agents that exhibit a high degree of aqueous solubility, new drug candidates are frequently highly lipophilic compounds. The aqueous environment of the blood provides a thermodynamically unfavourable environment for the disposition of such hydrophobic drugs. However, this limitation can be overcome by association with circulating lipoproteins. [3]

Drug Designing

Drug designing is the approach of finding drugs based on their targets and typically a drug target is a key molecule involved in a particular metabolism or signaling Pathway that is specific to a disease condition or Pathology or to be infectivity or survival of a microbial Pathogen. Some approaches attempt to stop the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. However these drugs would also have to be designed in such a way as not to affect any other important molecules that may be similar in appearance to the key molecules. [4]

Docking

Docking is a method which predicts the preferred orientation of one molecule to a record when bound to each other to form stable complex knowledge of the preferred orientations in turn may be used to predict the binding strength of association or binding affinity between two molecules. Docking is frequently used to predict the binding orientations of small molecules drug candidates to protein targets in order to in turn predict the affinity and activity of the small molecule. The receiving molecule that primarily binds to a small molecule or another protein or a nucleic acid called receptor. A molecule that forms the complementary partner in the docking process called ligand. [5]

Ligand

Acetohexamide is the first generation sulfonylurea medication used to treat diabetes mellitus type 2, particularly in people whole diabetes cannot be controlled by diet alone. Acetohexamide lowers blood sugar

by stimulating pancreas to secrete insulin and helps the body use insulin efficiently. The pancreas must produce insulin for this medication to work. It is an oral anti diabetic agent and is metabolized by the reductive conversion of the acetoxy group to a secondary alcohol metabolite. We tested whether reductase activity for acetohexamide can be found in human erythrocytes. Acetohexamide interact with other drugs such as alcohol, Beta blockers, cisapride, clofibrate, rifampin etc. If we missed to take dose, skip the missed dose and take only the next regularly scheduled dose. If acetoheaxamide will be a overdose it cause symptoms include hunger, nausea, anxiety, cold sweats, weakness, drowsiness and coma. [6]

MATERIALS AND METHODS

The Protein sequence has been retrieved from GenBank which is responsible for diabetes mellitus. The no. of amino acids present in IRAK protein has been identified. Then the lists of drugs for diabetes mellitus are retrieved and analyzed the hydrophobic activity for each drugs. Hydrophobic activity is calculated by ALOGPS tool. The Hydrophobic features are more responsible for all types of drugs. The distribution of the Log P and Log S values for each drug show the highest hydrophobic activity of the drug. The structure of the protein retrieved from Protein data bank and binding sites of the receptor was calculated by PROSITE tool.

RESULTS AND DISCUSSION

Hydrophobic Activity Of Various Drugs

ACETOHEXAMIDE

mol_N logP logS SMILES
mol_1 1.72 -3.83 CC(=O)c1ccc(cc1)S(=O)(=O)NC(=O)NC2CCCCC2

METFORMIN

mol_N logP logS SMILES
mol_1 -1.41 -1.76 CN(C)C(=N)N=C(N)N

PHENFORMIN

mol_N logP logS SMILES
mol_1 0.30 -3.02 c1ccc(cc1)CCN=C(N)N=C(N)N

MIGLITOL

mol_N logP logS SMILES
mol_1 -2.29 0.47 C1C(C(C(C(N1CCO)CO)O)O)O

TOLAZAMIDE

mol_N logP logS SMILES
mol_1 1.40 -3.01 Cc1ccc(cc1)S(=O)(=O)NC(=O)NN2CCCCCC2

GLICLAZIDE

mol_N logP logS SMILES
mol_1 1.52 -3.23 Cc1ccc(cc1)S(=O)(=O)NC(=O)NN2CC3CCCC3C2

VOGLIBOSE

mol_N logP logS SMILES
mol_1 -2.33 -0.15 C1C(C(C(C(C1(CO)O)O)O)O)NC(CO)CO

These drugs are treated for diabetes mellitus and the SMILE value for each drugs are retrieved from drug bank and submitted in to ALOGPS 2.1 tool. This tool provides the result with Log P and Log S values for each drug. **(Table 1)**

Table 1: Log P and Log S values for each drug

DRUG NAME	DRUG ID	Log P	Log S
ACETOHEXAMIDE	DB00414	1.72	-3.83
METFORMIN	DB00331	-1.41	-1.76
PHENFORMIN	DB00914	0.3	-3.02
MIGLITOL	DB00491	-2.29	0.47
TOLAZAMIDE	DB00839	1.4	-3.01
GLICLAZIDE	DB01120	1.52	-3.23
VOGLIBOSE	DB04878	-2.33	-0.15

In this table, we compared Log P and Log S values for each drug. The drug Acetohexamide has the highest Log P value (1.72) when compared with other drugs and it has log S value (-3.83).So, the hydrophobic activity of the Acetohexamide is higher. The drug Metformin has very low Log P value (-1.41) and Log S values (-1.76). The very low content of hydrophobic effect is present in Phenformin (0.3) but it has negative Log S value (-3.02). The Log P value is negative in Miglitol (-2.29) and Log S value has (0.47).This shows that hydrophobic activity is very less. The Log P value (1.4) and Log S value (-3.01) for Tolazamide shows that the activity of the drug is very less. The hydrophobic activity of the drug Gliclazide is higher due to Log P value (1.52) and it has Log S value (-3.23).The drug Voglibose has Log P value (-2.33) and it has Log S value (-0.15).

Figure 1 shows that the comparison of Log P values for each drug. From this graph, we can easily understand, the drugs Acetohexamide,Phenformin, Tolazamide and Gliclazide has Positive Log P values and Metformin,Tolazamide and Voglibose has Negative Log P Values Acetohexamide has highest Log P value (1.72) when compared with rest of the drugs.

We can get the knowledge about comparison of Log S values for each drug from **Figure 2**.From this graph, we can analysed the Log S values for the drugs involved in our analysis.Acetohexamide has very low Log S value (-3.83) but it has good hydrophobic effect.Miglitol is the only drug which has positive Log S value (0.47) and the rest of the drugs are negative Log s values.

From this **Figure 3** we can understand the comparison of Log P and Log S values for each drugs. The red colour shows that the Log P values and the Green colour shows that the Log S values for the drugs Acetohexamide, Metformin Phenformin, Miglitol, Tolazamide, Gliclazide and Voglibose respectively.

From the **Figure 4**, we can easily understand the docked position of the receptor and Ligand. The Green colour shows that the IRAK Protein and the Red colour shows that the Ligand Acetohexamide.

PROSITE RESULT ANALYSIS

HITS in IRAK Protein	3
Position of HITS in Protein	212-521,218-239,336-348
NP_BIND	218,226(ATP by similarity)
BINDING	239 (ATP by similarity)
ACT_SITE	340 Proton acceptor(By similarity)
Patterns	2
Profiles	1
Protein kinase domain Distinct Patterns	PS50011
Protein kinase ATP Binding region	PS00107
Serine/Threonine kinase active site region	PS00108

The Protein IRAK is responsible for diabetes mellitus and the sequence can be retrieved from NCBI and identifies the binding Site using PROSITE tool. This result shows that 3 hits present in the Protein including 2 patterns and 1 profile. It also shows that the positions of the binding sites present in the IRAK Protein (212-521,218-239,336-348).The position 218 and 226 is for NP_BIND and the proton acceptor site is present in the position 340.The ligand Acetohexamide binds in this position.

CONCLUSION

In this article, we have observed results that the drug acetohexamide has good hydrophobic effect based on Log P value and the IRAK Protein has 3 active sites. The docked results were identified by molecular docking method. The list of drugs are collected from drug bank which are used to treat Diabetes mellitus and identified the best drug based on hydrophobic activity. Hydrophobic features are valuable insight into the factors governing the pharmacological activity and potential toxicity of these compounds. In this review,we discussed the impact of hydrophobic drug–lipoprotein interactions on pharmacokinetics and biological activity of various hydrophobic compounds.Finally, we concluded that the ligand acetohexamide has the highest hydrophobic activity.

ACKNOWLEDGEMENT

The author would like to thank Mr.H.Karthick for their valuable suggestions and guidance. A grant from the Department of Bioinformatics, Thanthai Hans Roever College, Perambalur, India is gratefully acknowledged. The authors also thank the anonymous referees for their valuable and constructive comments.

Figure 1: Log P values for all drugs

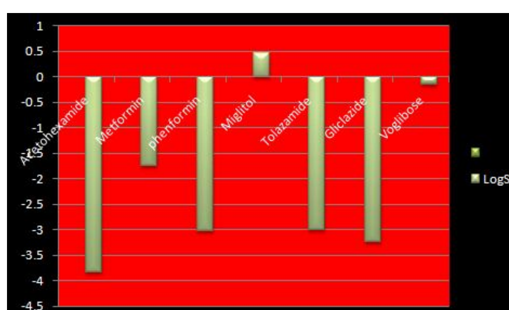

Figure 2:Log S values for all drugs

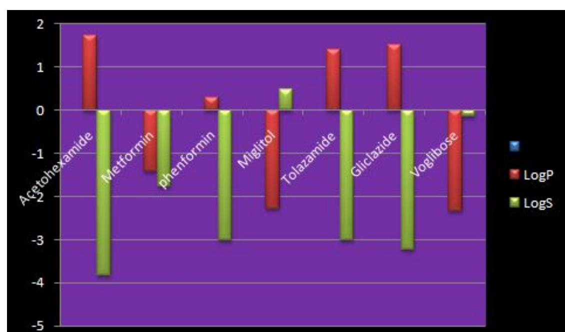

Figure 3:Log P and Log S values for all drugs

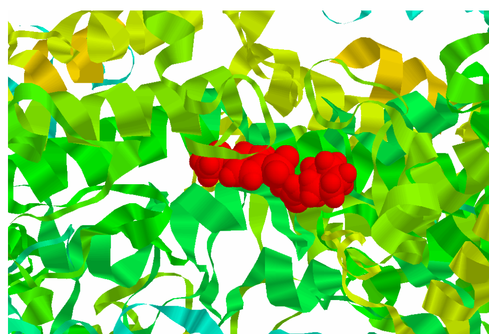

Figure 4: Screen Shot of Receptor and Ligand by Docking

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RESEARCH ARTICLE

Analysis of Phytochemicals by GC-MS on *Oldenlandia corymbosa* L.

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Received: 5 Aug 2010 Revised: 28 Nov 2010 Accepted: 1 Dec 2010

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ABSTRACT

Oldenlandia corymbosa L. (Rubiacea) is used against snake bite since ancient period and during labour also it used for strong uterine contraction. But its chemistry was not been explored still hence it has been take for phytochemical analysis by using Gas Chromatography coupled with Mass Spectrometry (GC-MS). There are 15 alkaloids were found out by using ethanolic extract of *O. corymbosa*, notably morpholin, quinazoline, erucamide alverine and dihydro pyrrole were found to present. Those alkaloids were already tested for its biological functions; the results suggest that plenty of those alkaloids available in this plant.

Key words: *O. corymbosa*, morpholin, quinazoline, erucamide alverine, dihydro pyrrole and GC-MS

INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by microorganisms, insects, and herbivores. Further, some of which may involve in plant odor (terpenoids), pigmentation (tannins and quinines), and flavor (capsacin). However, several of these molecules possess medicinal properties [1, 3, 14]. Manufacturing of Herbal and Ayurvedic products is simple and also good market demand for these products. According to the WHO (World Health Organization) as much as 80% of the world's population relies on traditional medicine. With increased concerns about rising health care costs, some governments are encouraging the use of indigenous form as of medicines rather than expensive drugs. This has been a strong driver for resuscitation of herbal and Ayurvedic medicine in the country. Traditional treatment with

Ayurvedic and other herbal medicines is well established and widely acknowledged to be safe effective [2,8,9].

The investigation revealed that, the traditional healers used 85 species (including Corymbosa) of plants distributed in 76 genera belonging to 41 families to treat various diseases. *Oldenlandia corymbosa* L. is used for arresting blood bleeding [1]. *Oldenlandia corymbosa* L. of Thai origin, a member of the family Rubiaceae, is widely distributed in tropical regions of Asia. The decoction of whole plants used in tradition Thai medicine for antipyretic purpose to decrease body temperature. Pharmaceutically, the anti-inflammatory, antioxidant, and hepatoprotective properties of plant extracts have been reported. This plant is well known to contain mainly iridoid glucosides. However, there are a few reports on the presence of other types of compounds, particularly anthra quinones and triterpenoids [3, 4]. γ-Sitosterol and the triterpene acids, oleanolic acid and ursolic acid, have been shown to be present in the Indian medicinal plant Oldenlandia corymbosa L. Evidence is presented to show that the plant does not contain any alkaloid [5].

Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC/MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

MATERIALS AND METHODS

Alkaloids cannot be identified or quantified by a single method because these are highly heterogeneous chemically and there are so many of them. In general, it is difficult to identify an alkaloid from a new plant source without knowing approximately what type of alkaloid is likely to be found there. Also, because of the wide range of solubility and other properties of alkaloids, any general screening procedure for alkaloids in plants may fail to detect particular compounds [6].

Preparation of plant extract for phytochemical analysis

About 250g of dry powder of *Oldenlandia corymbosa* L. was extracted with diethyl ether, acetone and ethanol at $60^{o}c$ to $80^{o}c$ by continuous hot percolation using Soxhlet apparatus. The extraction was filtered and kept in over at $50^{o}c$ for 24 hours to evaporate the extracts from them. A greenish black waxy residue was obtained. These extracts were used for phytochemical analysis qualitatively.

Preparation of plant extract for alkaloid analysis by GC-MS

Plants (whole plant) were collected, cleaned with tap water and dried under shade. The dried parts of medicinal plants were ground well to find powder. The ground sample was made alkaline with 39% ammonia and extracted with chloroform at room temperature for a total period of 24 hrs and then the extract was partitioned between 5% HCl and chloroform. The aqueous phase was made alkaline again with ammonia and partitioned between water and chloroform. Finally chloroform was totally evaporated from the organic phase to form the alkaloid powder [6].

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extract was undertaken using standard qualitative methods as described by various authors [7, 18, 19, 20]. The plant extracts were screened for the presence of biologically active compounds like alkaloids, flavonoids, glycosides, carbohydrates, phytosteroids and fatty acids, proteins, phenolics, tannins and saponins.

RESULTS AND DISCUSSION

Phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, phytosterols, fixed oil and fats, proteins, phenolic compounds, and saponins of *Oldenlandia corymbosa* were analyzed by qualitatively and reported in **Table - 1.** Gas chromatograph of Alkaloids of *Oldenlandia corymbosa L.* was recorded, each peak in chromatograph implies the presence of group of Alkaloids. Mass spectrograph of Alkaloids of *Oldenlandia corymbosa L.* was recorded, each peak in spectrograph implies the presence of individual Alkaloids according to their charge to mass ratio (e/m) which are indicated in figures 1and 2 [10,11,12,13].

The following alkaloids were individually analyzed by GC-MS technique

Table: 1 Shows Phytochemical Constituents

Sl.No.	Secondary metabolites	Ethanol	Diethyl ether	Acetone
01	Alkaloids	+++	+	+
02	Flavonoids	++	+	++
03	Terpenoids	+++	+++	+++
04	Carbohydrates	+++	+++	+++
05	Glucosides	++	++	++
06	Phenolics	++	++	++
07	Phytosterols	+++	++	+++
09	Proteins	+++	+	++

Oldenlandia corymbosa L. possess 15 alkaloids (**Table 2**). So ethanolic extract of this plant exhibit strong antioxidant potential, radical scavengers, nitric oxide inhibitors, they are also found to have weakly cytotoxic and possess moderate antibacterial properties [15,16,17].

Table: 2 Alkaloids individually analyzed by GC-MS technique

Sl.No	Alkaloid	Class	Molecular Weight
1	Dimethyl amino Morpholin	True alkaloid	315
2	Formyl butanamide	Proto alkaloid	171
3	Heptane diamine	Proto alkaloid	186
4	Quinazoline	True alkaloid	268
5	Benzothiopyran-4-one	True alkaloid	269
6	Dihydro pyrrole	True alkaloid	269
7	Thiomethyl pyririnyl ketene	True alkaloid	268
8	Triazol-3-amine	Protoalkaloid	98
9	Lycorenan-7-one	True alkaloid	130
10	Alverine	True alkaloid	281
11	Dextropropoxyphene	Pseudo alkaloid	339
12	Azacyclohexadecane	Protoalkaloid	284
13	Erucamide	True alkaloid	337
14	Piperidyloxo pentanoic acid	True alkaloid	266
15	Diazene	Protoalkaloid	150

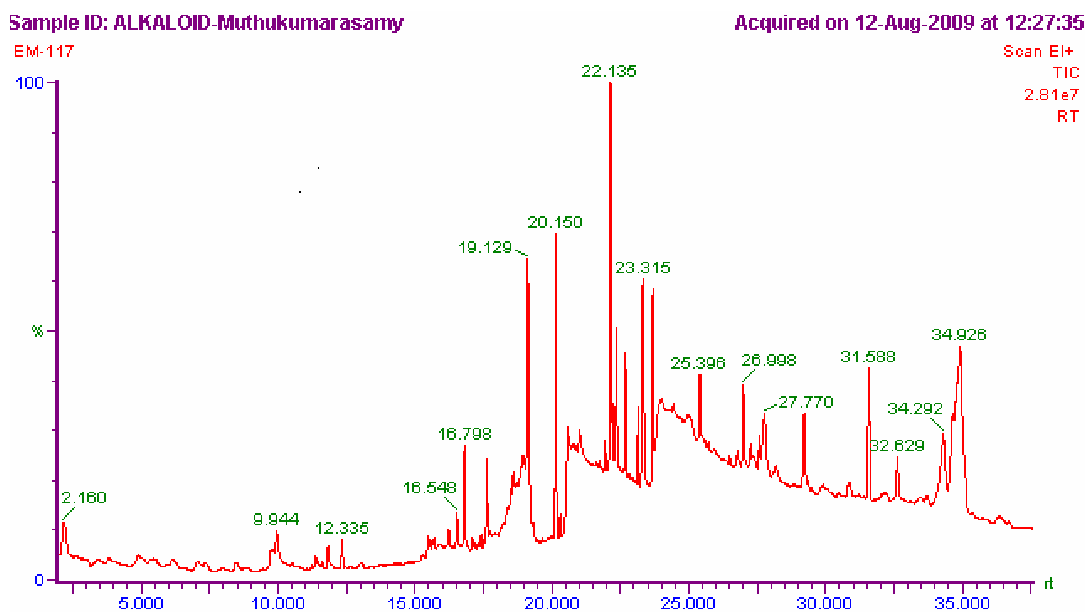

Figure: 1 Chromatograph of Alkaloids of *Oldenlandia corymbosa* L.

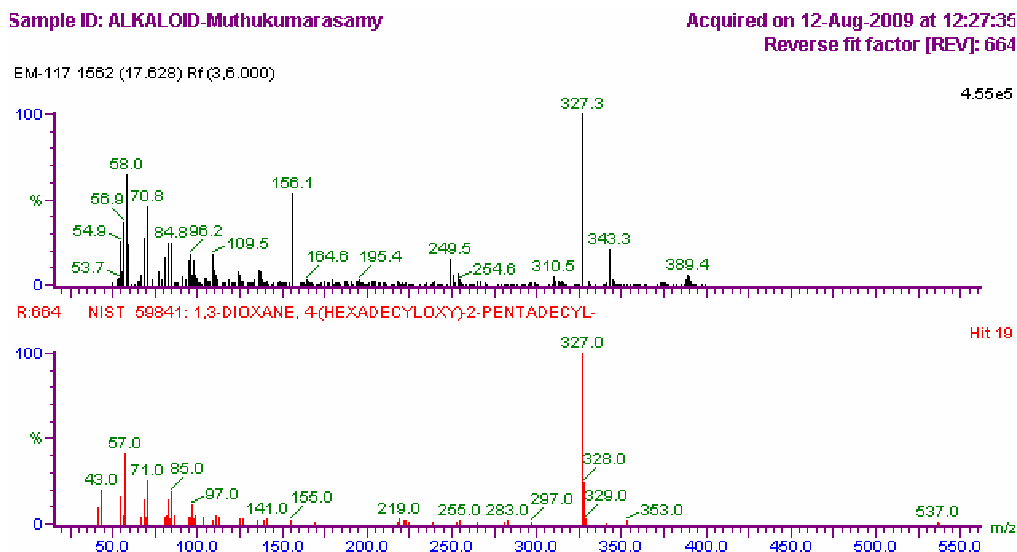

Figure: 2 Mass spectrograph of Alkaloids of *Oldenlandia corymbosa* L.

CONCLUSION

It is concluded that Oldenlandia corymbosa has above said alkaloids a lot, it is known that alkaloids exerts antioxidant activity, so it may have anticancer effects too.

ACKNOWLEDGEMENT

Our gratitude to Dr.Vellaiyan, Department of Botany, JJ College of Arts and Science, Pudukkottai for giving identification and authentication on systematic position of this plant.

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Indian Journal Of Natural Sciences ISSN: 0976 - 0997
Vol 1 / Issue 3 / December 2010 © IJONS

REVIEW ARTICLE

Bacopa monnieri (L.) Pennell. - an Overview

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Received: 18 Oct 2010 Revised: 6 Nov 2010 Accepted: 28 Nov 2010

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ABSTRACT

Developing countries like India is having a rich heritage of medicinal plants. Based on the natural resources available, many of the medicinal herbs being used effectively for their therapeutic potentials by the tribal and most of the rural and some of urban societies too. Recently the diseases like cancer and Diabetes found to be much higher ratio among Indian population, based on known and unknown factors too. Among the medicinal plants used in the management of cancer, the plant *Bacopa monnieri* (L.) Pennell also one reputed plant used in the cancer studies. *Bacopa monnieri* (L.) Pennell is famous for its antistress, immunomodulatory; cognition-facilitating, anti-inflammatory and anti-aging effects produced by it in experimental animals and in clinical situations and may justify further investigation of its other beneficial properties. Moreover, this experimental evidence suggests that, *Bacopa monneri* may be useful in the treatment of human pathologies in which free radical production plays a key role. This review highlights the details about the plant and its therapeutic usefulness in the management of cancer and related complications.

Keywords: *Bacopa monnieri* (L.) Pennell; antioxidant; cancer, medicinal uses.

INTRODUCTION

Bacopa monnera, also called as *Bacopa monneri*, it has been used in the Indian system of medicine (Ayurvedic) for centuries. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration [1], and to provide relief to patients with anxiety or Convulsive disorders [2]. The plant has also been used in India and Pakistan as a cardio tonic, digestive aid, and to improve respiratory function in cases of bronchoconstriction [3]. Recent research has focused primarily on Bacopa's cognitive-enhancing effects, specifically memory, learning, and concentration and results support the

traditional Ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of Bacopa. Bacopa's antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer.

Description: *Bacopa monnieri*(L.) Pennell, a member of the Scrophulariaceae family, is a small, creeping plant with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally in wet soil in condition, shallow water, and marshes. The herb can be found at elevations from sea level the altitudes of 4,000 feet, and is easily cultivated if adequate water is available. Flowers and fruit appear in summer and the entire plant is used medicinally [2, 4].

Bacopa monnieri (L.) Pennell.

Scientific Classification

Kingdom : Plantae

Order : Lamiales

Family : Scrophulariaceae

Genus : Bacopa

Species : *monnieri*(L.) Pennell.

Phyto constituents: Compounds responsible for the pharmacological effects of *Bacopa monnieri* (L.) Pennell include alkaloids : (Hydrocotyline, Brahmine, Herpestine) , saponins (d-mannitol , Monnierin Hersaponin , Bacoside A and Bacoside B) And sterols. Other active constituents have since been identified, including betulic acid, stigmastarol, beta-sitosterol, as well as numerous bacosides and bacopasaponins. The constituents responsible for Bacopa's cognitive effects are bacosides A and B [5-9].

Mechanism of action in *Bacopa monnieri* (L.) Pennell: The triterpenoid saponins and their bacosides are responsible for Bacopa's ability to enhance nerve impulse transmission. The bacosides aid in repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity, and ultimately nerve impulse transmission [9]. Loss of cholinergic neuronal activity in the hippocampus is the primary feature of Alzheimer's disease [10]. Based on animal study results, bacosides appear to have antioxidant activity in the hippocampus, frontal cortex, and striatum [11]. Animal research has shown Bacopa extracts modulate the expression of certain enzymes involved in generation and scavenging of reactive oxygen species in the brain [12]. *In vitro* research has shown Bacopa exerts a protective effect against DNA damage in astrocytes [14] and human fibroblasts [13]. In animals Bacopa has a relaxant effect on pulmonary arteries, aorta, trachea, and ileal and bronchial tissue, possibly mediated by inhibition of calcium- ion influx into cell membranes [14]. Bacopa appears to stabilize mast cells *in vitro* [15], and possesses anti-inflammatory activity via inhibition of prostaglandin synthesis and lysosomal membrane stabilization [16]. *In vitro* research suggests an anticancer effect for Bacopa extracts, possibly due to inhibition of DNA replication in cancer cell lines [17].

Anticancer activity in *Bacopa monnieri* (L.) Pennell: Bhakuni, D et al [18] was evaluated the alcoholic extract of *Bacopa* has been shown to possess anticancer activity against Walker carcinosarcoma 256 in rats, growth-inhibitory effects on Sarcoma 180 cultures10, activity affecting avoidance response in rats. Elangovan V *et al* [19] was evaluated *In vitro* research demonstrated Bacopa saponin fractions have cytotoxic activity for sarcoma-180 cells. It is thought this might be due to Bacopa's inhibition of DNA replication in the cancerous cell line. Research in humans may be indicated. Prashanth D'Souza *et al* [20] was screened

about the Successive petroleum ether, chloroform, ethanol and water extracts, a saponin rich fraction (SRF) and bacoside A isolated from *Bacopa monnieri* (L.) Pennell were tested for brine shrimp lethality. Successive ethanol extracts and SRF showed potent activity. Bacoside A showed the maximum activity with a LC_{50} of 38.3 μg/mL. The results confirmed the previous reports of an anticancer effect of *Bacopa monnieri* (L.) Pennell and suggest bacoside A as the active constituent. M.Mastan *et al* [21] *was screened Cytosine arabinoside (1-β - arabinofuranosylcytosine; Ara-C)* is the most important anti metabolite chemotherapeutic drug used for acute leukemia. Panneerselvam Janani et al were evaluated strong anti-oxidant and hepatoprotective effects of bacoside A (BA) against carcinogen. Nevertheless the effect of BA on the activities and expression of MMP-2 and MMP-9 during hepatocellular carcinoma is not yet recognized. Results of gelatin zymography study showed that BA co-treatment significantly decreased the activities of MMP-2 and MMP-9, which is increased during hepatocellular carcinoma. Further immunoblot analysis showed decreased expression of MMP-2 and MMP-9 in rats co-treated with BA compared to DEN-induced hepatocellular carcinoma.

Drug/Botanical Interactions: Bacopa has been noted in animal models to decrease the toxicity of morphine and phenytoin. It has also been shown, albeit inconsistently, to have a slight sedative effect, so caution is advised in combination with other known sedatives. Also, since it appears to stimulate T4 activity in animals at high doses, it is theorized it may potentiate the activity of thyroid-stimulating drugs or inhibit the effect of thyroid-suppressant drugs.

Side Effects and Toxicity: Therapeutic doses of Bacopa are not associated with any side effects, and Bacopa has been used safely in Indian system of medicine for century of years. A double-blind, placebo controlled clinical trial of healthy male volunteers investigated the safety of pharmacological doses of isolated bacosides over a four-week period. Concentrated bacosides given in single (20-30 mg) and multiple (100-200 mg) daily doses were well tolerated and without adverse effects. The LD50 of Bacopa extracts administered orally to rats was 5 g/kg for aqueous extracts and 17 g/kg of the alcohol extract. Neither extract resulted in gross behavioral changes at this concentration.

CONCULSION

Bacopa monnieri (L.) Pennell or Brahmi is an Indian herb that has been in use for a very long time for its wonderful benefits. It is known for its ability to improve brain function, boost memory, and aid in the management of several diseases. It is considered to be a neurotonic due to its value in increasing memory and concentration. It can also be used as an alternative treatment to several disorders like epilepsy, depression, anxiety, and ADHD. It has long been a part of the Ayurveda, the Indian folk medicine system. Bacopa monnieri contains apigenin and luteolin, two flavonoids that make good antioxidants. It also contains two saponins called bacopaside I and II.

Saponins are known to lower blood cholesterol levels, decrease the risk of cancer, and also strengthen the immune system. Flavonoids and saponins are essential to deliver the health benefits associated with *Bacopa monnieri* (L.) Pennell and helps give the body nutrients.When it comes to the memory enhancing property of bacopa monnieri, it can actually be attributable to the two active molecules called bacopasides. Bacopasides contain properties known to improve memory, enhance focus and concentration, and aids with learning new tasks.

In fact, regular intake of bacopa increases protein kinase activity as well as protein production in the brain cells, which play a role in learning and memory. Bacopa also helps with the repair of damaged neurons in the brain, helping enhance brain function and boosting memory. Bacopa is also of benefit to those with Alzheimer's. It inhibits breakdown of cholinesterase, a neurotransmitter that is primarily affected in the disorder. For those with asthma and bronchitis, bacopa is also valuable because of its relaxing effect on constricted bronchioles. It also has a potential to control allergies and asthma because of its ability to stabilize mast cells. Some studies also suggest that bacopa may have anti-cancer properties due to its ability

to hinder DNA replication of cancer cells. This herb may also be helpful for sufferers of irritable bowel syndrome or other conditions with intestinal spasms. It may also be of benefit for those with thyroid problems like hypothyroidism due to its stimulating effect on thyroid function. You can take *Bacopa monnieri* (L.) Pennell alone as an herbal extract but a better way is to take multivitamins that has bacopa as part of the ingredients. That way, you can be sure that you get health benefits from other vitamins and minerals as well. Just make sure that the brand of vitamins you will buy is made from all natural ingredients like green tea extracts and CoQ10 to help give you a healthy body.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of SASTRA University.

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RESEARCH ARTICLE

Phytochemical Investigations and Screening of Antihyperlipidemic of *Achyranthes aspera* Linn. and *Achyranthes bidentata* Blume.

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Received: 20 Oct 2010 Revised: 6 Nov 2010 Accepted: 30 Nov 2010

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ABSTRACT

Achyranthes aspera Linn. and *Achyranthes bidentata* Blume. two plant materials were collected from different parts of Nilgiri district of Tamilnadu and authenticated. Physicochemical parameters were determined. Fluorescence analysis was carried out for the plant powder and their extracts. The dried and powdered leaves and seeds of plants were extracted with water and 50% ethanol through cold maceration. Alcohol soluble extractive and water soluble extractive values were determined. The phytochemical studies of the plant extracts showed the presence of alkaloids, glycosides, triterpenoids, saponins, flavonoids and mucilage. These results gave clues regarding the presence of some particular phytoconstituents in the respective plant extracts. The *in vivo* screening for antihyperlipidemic activity with triton induced hyperlipidemia and high fat diet induced hyperlipidemia model showed that both aqueous and 50% ethanol extracts of plants at a dose of 200 mg/kg exhibited significant activity against hyperlipidemia. Antihyperlipidemic activity of the various extracts may be attributed to the presence of triterpenoids, saponins, and flavonoids. From these studies, it can be concluded that both the plants are endowed with significant antihyperlipidemic activity.

Keywords: *Achyranthes aspera* Linn., *Achyranthes bidentata* Blume., hyperlipidemia, triterpenoids, saponins, and flavonoids

INTRODUCTION

Hyperlipidemia, the elevation of lipid concentration in plasma, is the manifestation of a disorder in the synthesis and degradation of plasma lipoproteins. Primary type hyperlipidemia can be treated with drugs but the secondary type originating from diabetes, renal lipid necrosis or hypothyroidism demands the treatment of original disease rather than hyperlipidemia [1]. Levels between 200 and 240 mg/dL indicate moderate risk, and levels surpassing 240 mg/dL indicate high risk. While their role in heart disease is not entirely clear, it appears that as triglyceride levels rise, levels of good cholesterol fall. It is the complex

interaction of these three types of lipids that is thrown off when a person has hyperlipidemia. High cholesterol is characterized by elevated levels of LDL cholesterol, normal or low levels of HDL cholesterol, and normal or elevated levels of triglycerides. According to World Health organization (WHO) 2002, almost one fifth (18 %) of global stroke events (mostly nonfatal events) and about 56 % of global heart disease are attributable to total cholesterol levels above 3.2 mmol/l. This amounts to about 4.4 million deaths (7.9 % of the total) and 2.8 % of the global disease burden.

MATERIALS AND METHODS

Collection and authentication of plant materials

The plants *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume [2] (family: Amaranthaceae) are widely found throughout India up to a height of 1200m. In TamilNadu, these are found in Nilgiri district and in Erode district. Were identified by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Chennai, a botanist who authenticated the plant with available literature

Preparation of the extracts

The plant material which was powdered and stored was used for extraction. A weighed quantity of each of the plant powdered material was extracted by cold maceration with 50% ethanol for 72 hr with intermediate heating at 40°C one time in a day. The extract was filtered using Whatmann filter paper and then the filtrate was concentrated under reduced pressure and controlled temperature (40°-50°C). The marc was dried and weighted. The marc was again extracted with water by cold maceration for 72 hrs to yield aqueous extract.

Phytochemical Screening

Phytochemical analyses for the above the plant extracts were performed and the phytoconstituents reported.

Antihyperlipidemic screening

Healthy adult male albino rats of Wistar strain weighing between 180-220g were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India for the screening of antihyperlipidemic activity of the plant extracts. The animal were housed in polypropylene cages in adequately, well ventilated room and maintained under standard environment conditions (22-28°C, 60-70% relative humidity, 12h dark/light cycle). The animals were fed with standard rat feed pellets (Amurth Rat Feed, Nav Maharashtra Chakan Oil Mills Ltd., Pune) and water ad libitum (Aquaguard filter water). The study was approved by the institutional animal ethics committee (approval no: JSSCP/IAEC/Ph.D/Ph.Cology/ 02/2008-09).

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=3) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for a overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. It mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.

Preparation of test solution

The aqueous and ethanolic extracts of Achyranthes *aspera* Linn and *Achyranthes bidentata* Bl leaf and seeds were suspended in 0.3% CMC separately, and administered orally at 100 mg/kg and 200 mg/kg body weight.

Standard drug

Atorvastatin (2mg/kg) was suspended in 0.3% CMC and administered orally to rats.

Triton induced hyperlipidemia [3]

Grouping of animals

The experimental design of the investigation was carried out in 19 groups with six animals in each group in the following regimen;

Group I	Received 0.3% w/v carboxy methyl cellulose (CMC) orally for one week.
Group II	Received triton (250 mg/kg b.w) i.p route
Group III	Received atorvastatin (2 mg/kg) for 7 days in 0.3% CMC, Orally
Group IV	Received aqueous leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group V	Received aqueous leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group VI	Received ethanolic leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group VII	Received ethanolic leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group VIII	Received aqueous seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group IX	Received aqueous seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group X	Received ethanolic seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group XI	Received ethanolic seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group XII	Received aqueous leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XIII	Received aqueous leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XIV	Received ethanolic leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XV	Received ethanolic leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XVI	Received aqueous seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XVII	Received aqueous seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XVII	Received ethanolic seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XIX	Received ethanolic seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

On 8th day for the overnight fasted rats, triton was administered at 250 mg/ kg, by intraperitoneal route. The blood samples were collected after triton administration at 0h and 24h. These were centrifuged for 15 minutes at 3000 rpm and plasma was separated. Plasma samples were used for the estimation of cholesterol, triglyceride and HDL cholesterol, using Merck kits in auto analyzer (Microlab 200, Merck).

High fat diet induced hyperlipidemia [4]

Grouping of animals

The experimental design of the investigation was carried out in 18 groups with six animals in each group and carried out in the following regimen;

Group I	Received normal diet
Group II	Received cholesterol rich diet for 28 days
Group III	Received aqueous leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group IV	Received aqueous leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group V	Received ethanolic leaf extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group VI	Received ethanolic leaf extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group VII	Received aqueous seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group VIII	Received aqueous seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group IX	Received ethanolic seed extract of *Achyranthes aspera* Linn (100 mg/kg b.w) for 7 days, orally
Group X	Received ethanolic seed extract of *Achyranthes aspera* Linn (200 mg/kg b.w) for 7 days, orally
Group XI	Received aqueous leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XII	Received aqueous leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XIII	Received ethanolic leaf extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XIV	Received ethanolic leaf extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XV	Received aqueous seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XVI	Received aqueous seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally
Group XVII	Received ethanolic seed extract of *Achyranthes bidentata* Bl (100 mg/kg b.w) for 7 days, orally
Group XVIII	Received ethanolic seed extract of *Achyranthes bidentata* Bl (200 mg/kg b.w) for 7 days, orally

The animals in group II to XVIII were fed with atherogenic diet (high fat diet) consisting of rat chow (67g), cholesterol (1.5g), milk powder (8g), salt (2g), coconut oil (5ml) and multiple vitamin (0.5g). The control animals (group I) was fed with normal diet for 28 days. Blood sample were collected and plasma was separated. This was used for estimation of cholesterol, triglyceride and HDL cholesterol, LDL and VLDL cholesterol. The rats were sacrificed after the collection of blood samples and the aorta and liver were excised immediately for histopathological examination.

RESULTS AND DISCUSSIONS

Table 1: Yield and nature of the extracts

S.No	Plant material	Quantity used for extraction in grams	Type of the extract	Colour of the extract	Yield	
					G	%
1	*Achyranthes aspera* Linn leaf	900	50% ethanol	Dark green	122	13.63
2	*Achyranthes aspera* Linn leaf	760	Aqueous	Dark brown green	147	19.34
3	*Achyranthes aspera* Linn seed	800	50% ethanol	Dark brown green	130	16.25
4	*Achyranthes aspera* Linn seed	620	Aqueous	Dark brown green	126	20.28
5	*Achyranthes bidentata* Blume leaf	900	50% ethanol	Dark green	127	14.11
6	*Achyranthes bidentata* Blume leaf	730	Aqueous	Dark brown green	147	20.13
7	*Achyranthes bidentata* Blume seed	800	50% ethanol	Dark brown green	136	17
8	*Achyranthesbidentata* Blume seed	610	Aqueous	Dark brown green	129	21.14

Phytochemical studies

Organoleptic characters of *Achyranthes aspera* and *Achyranthes bidentata* leaf

The leaf powder of *Achyranthes aspera* and *Achyranthes bidentata* is pale green in colour with characteristic odour and no characteristic taste. The powder was coarse in appearance and when triturated with water it was non sticky in nature. The powder on shaking with water gave foam like froth and no oil stain was found when the powder was pressed between filter papers for 24 hours.

Table 2: Organoleptic character of Achyranthes *aspera* leaf raw powdered material

Powder character	
Colour	Pale green
Appearance	Coarse powder
Odour	Characteristics
Taste	No Characteristics

Treatment	Observation
Powder triturate with water	Non sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 3: Organoleptic character of Achyranthes *bidentata* leaf raw powdered material.

Powder character	
Colour	Pale green
Appearance	Coarse powder
Odour	Characteristics
Taste	No Characteristics
Treatment	**Observation**
Powder triturate with water	Non sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Organoleptic characters of *Achyranthes aspera* and *Achyranthes bidentata* seed

The seed powder of *Achyranthes aspera* and *Achyranthes bidentata* is pale brown in colour with characteristic odour and mucilaginous taste. The powder was fine in appearance and when triturated with water it was sticky in nature. The powder on shaking with water gave foam like froth and no oil stain was found when the powder was pressed between filter papers for 24 hours. A preliminary organoleptic character of both the powdered plant material was studied and the results are shown in **Table 4**.

Table 4: Organoleptic character of Achyranthes *aspera* seed raw powdered material

Powder character	
Colour	Pale brown
Appearance	Fine powder
Odour	Characteristic
Taste	Mucilaginous
Treatment	Observation
Powder triturate with water	Sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 5: Organoleptic character of Achyranthes *bidentata* seed raw powdered material.

Powder character	
Colour	Pale brown
Appearance	Fine powder
Odour	Characteristic
Taste	Mucilaginous
Treatment	**Observation**
Powder triturate with water	Sticky
Powder shaken with water	Foam like froth
Powder pressed between filter paper for 24 hrs	No oil stain

Table 6: Qualitative phytochemical analysis of raw powder and extract of plant leaf

Constitients	**Raw powder**				**50 % Ethanol extracts of *Achyranthes aspera* leaf**	**Aqueous extract of *Achyranthes aspera* leaf**	**50 % Ethanol extracts of *Achyranthes bidentata* leaf**	**Aqueous extract of *Achyranthes Bidentata* leaf**
	***Achyranthes Aspera* leaf**		***Achyranthes bidentata* leaf**					
	Alc	**Aq**	**Alc**	**Aq**				
Alkaloids	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+
Gum	-	-	-	-	-	-	-	-
Fixed oils	-	-	-	-	-	-	-	-
Mucilage	+	+	+	+	+	+	+	+

Alc-Alcoholic, Aq-Aquous,

Table 7: Qualitative phytochemical analysis of raw powder and extract of plant seed

Constitients	Raw powder				50 % Ethanol extracts of *Achyranthes aspera* seed	Aqueous extract of *Achyranthes aspera* seed	50 % Ethanol extracts of *Achyranthes bidentata* seed	Aqueous extract of *Achyranthes Bidentata* seed
	Achyranthes Aspera seed		*Achyranthes bidentata* seed					
	Alc	Aq	Alc	Aq				
Alkaloids	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+
Gum	-	-	-	-	-	-	-	-
Fixed oils	+	-	+	-	+	+	+	-
Mucilage	+	+	+	+	+	+	+	+

Alc-Alcoholic, Aq-Aquous,

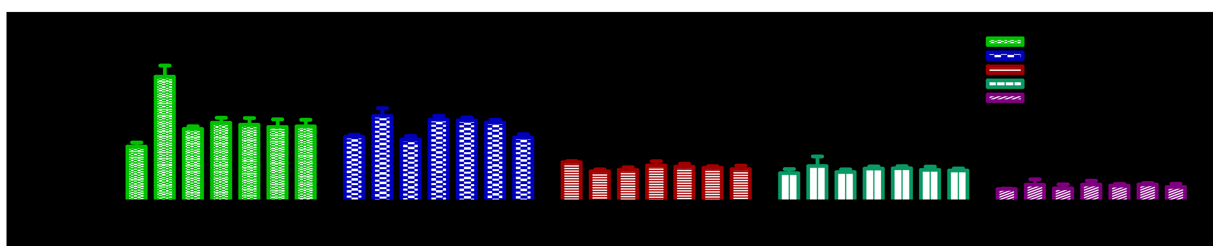
Triton induced hyperlipidemia

Table 8: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	26.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	7.56 ±2.97	20.5 ±4.7###
Atorvastatin (G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.2***	31.13 ±3.6	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	7.99 ±1.98	11.4 ±3.5***
AA aqueous leaf extract (100)(G4)	64.76 ±3.1	68.63 ±4.32***	55.34 ±1.43	71.37 ±3.3	34.86 ±1.23	31.38 ±3.65*	24.72 ±1.69	28.63 ±1.92*	12.34 ±1.94	14.7 ±3.3*
AA aqueous leaf extract (200)(G5)	53.76 ±4.86	66.89 ±5.76***	44.65 ±3.39	70.75 ±2.5	33.34 ±2.34	30.25 ±2.55	21.94 ±1.86	28.89 ±1.76*	9.65 ±1.39	13.75 ±1.5**
AA 50% ethanol leaf extract (100)(G6)	57.87 ±5.5	65.46 ±6.6***	39.54 ±3.54	68.86 ±2.54	33.24 ±1.76	29.39 ±1.32	27.87 ±2.15	27.46 ±2.86*	9.54 ±2.32	14.8 ±1.2*
AA 50% ethanol leaf extract (200)(G7)	60.76 ±7.52	64.96 ±5.7***	38.66 ±5.85	55.86 ±2.73*** €€€	32.56 ±2.93	28.24 ±3.00	19.75 ±2.58	26.96 ±1.76**	8.66± 106	12.6 ±2.7***

*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G4, G5, G6 and G7
€€€-P<0.001 when compared G6 vs G7



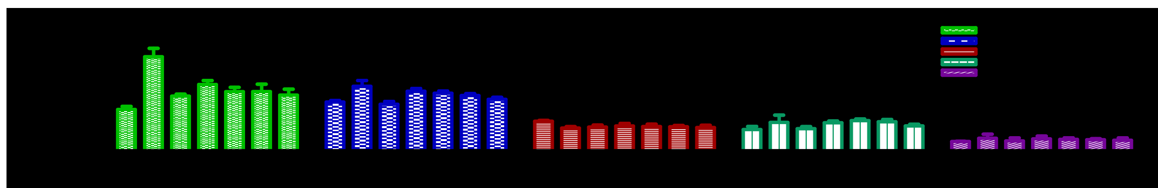
G1=Control, G2=Triton, G3=Atorvastatin, G4=AA aqueous leaf extract(100), G5=AA aqueous leaf extract(200), G6= AA 50% ethanol leaf extract(100), G7= AA 50% ethanol leaf extract(200)

Figure 1: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Table 9: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70$^{\#\#\#}$	44.53 ±2.16	74.62 ±6.7$^{\#\#\#}$	35.4 ±2.32	26.36 ±1.57$^{\#\#\#}$	22.30 ±3.87	35.87 ±8.36$^{\#\#\#}$	12.56 ±2.97	14.5 ±4.7$^{\#\#\#}$
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	7.99 ±1.98	11.4 ±3.1***
AA aqueous seed extract(100)(G8)	62.75 ±2.89	76.68 ± 4.53***	58.53 ±1.46	68.93 ±3.1	44.78 ±1.53	28.69 ±2.97	26.67 ±1.57	32.47 ± 1.96	11.25 ±1.84	13.8 ±3.2**
AA aqueous seed extract(200)(G9)	61.67 ±4.86	68.45 ±4.96***	46.79 ±3.34	66.68** ±2.5	38.46 ±2.33	28.15 ±2.63	22.26 ±1.97	34.99 ±1.83	10.43 ±1.29	13.2 ±1.8**
AA 50% ethanol seed extract(100)(G10)	58.43 ±5.5	68.48 ±8.6***	43.44 ±3.83	63.96 ±2.2***	35.76 ±5.74	27.88 ±1.35	22.87 ±2.86	33.59 ±2.64	9.34 ±2.3	12.8 ±1.2***
AA 50% ethanol seed extract(200)(G11)	56.74 ±6.22	64.46 ±6.82***	45.73 ±6.85	59.53 ±2.3***	33.92 ±2.71	26.96 ±2.56	18.57 ±2.88	28.86 ±1.86*	8.69 ±1.3	12.2 ±2.7***

Values are expressed as mean±S.D; n=6.$^{\#\#\#}$-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G8, G9, G10 and G11



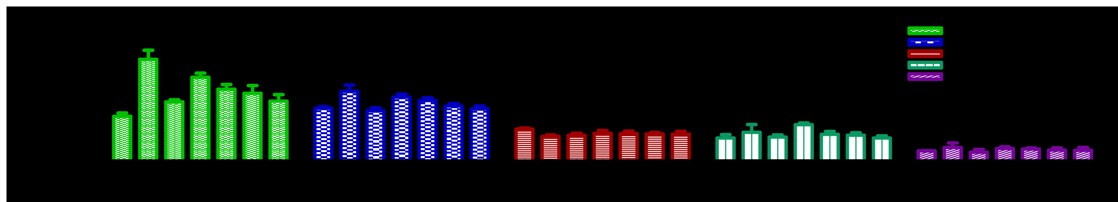
G1=Control, G2=Triton, G3=Atorvastatin, G8=AA aqueous seed extract(100), G9=AA aqueous seed extract(200), G10= AA 50% ethanol seed extract(100), G11= AA 50% ethanol seed extract(200)

Figure 2: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Table 10: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	26.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	7.56 ±2.97	14.5 ±4.7###
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	4.99 ±1.98	9.4 ±3.1***
AB aqueous leaf extract(100)(G12)	61.76 ±2.15	89.63 ±4.3***	42.34 ±2.13	68.37 ±3.2***	33.86 ±1.86	29.66 ±2.96	20.76 ±1.86	38.89 ±1.76	6.68 ±1.35	13.4 ±1.8**
AB aqueous leaf extract(200)(G13)	59.76 ±5.01	76.89 ±5.04***	40.65 ±3.17	64.75 ±2.9***	38.67 ±2.39	29.25 ±2.68	18.84 ±2.23	28.56 ±3.08*††	5.89 ±1.85	12.8 ±1.2***
AB 50% ethanol leaf extract(100)(G14)	56.87 ±5.5	72.46 ±8.4***	38.54 ±3.95	58.86 ±2.7***	33.63 ±1.76	28.88 ±1.56	18.57 ±1.67	27.57 ±2.65*	5.75 ±1.23	11.7 ±2.3***
AB 50% ethanol leaf extract(200)(G15)	54.76 ±6.22	64.06 ±6.94***	36.66 ±5.05	55.86 ±3.0***	34.9 ±2.6	28.76 ±2.95	16.92 ±0.95	24.64 ±2.30***	5.21 ±1.98	11.4 ±3.1***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G12, G13, G14 and G15
††-P<0.01 when compared G12 vs G13



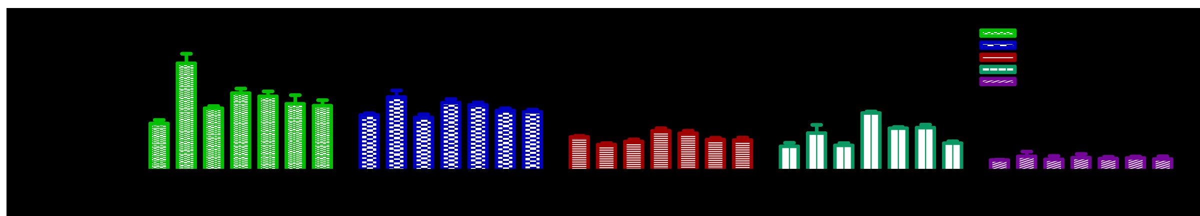
G1=Control, G2=Triton, G3=Atorvastatin, G12=AB aqueous leaf extract(100), G13=AB aqueous leaf extract(200), G14= AB 50% ethanol leaf extract(100), G15= AB 50% ethanol leaf extract(200)

Figure 3: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Table 11: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	28.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	26.56 ±2.97	14.5 ±4.7###
Atorvastatin (G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	29.53 ±2.14	15.92 ±0.95	25.64 ±2.30***	7.99 ±1.98	11.4 ±3.1***
AB aqueous seed extract(100) (G16)	58.72 ±1.45	78.64 ±4.57***	42.53 ±1.46	68.93 ±3.7	34.78 ±1.8	40.46 ±2.52	28.67 ±1.45	28.47 ±1.48**	23.25 ±1.2	13.2 ±3.7**
AB aqueous seed extract(200) (G17)	58.67 ±3.86	75.35 ±4.96***	40.79 ±3.34	66.68 ±2.5**	34.46 ±2.33	37.86 ±2.55	18.26 ±1.43	22.99 ±1.24***	21.43 ±1.48	12.2 ±1.8***
AB 50% ethanol seed extract(100) (G18)	57.43 ±5.5	67.84 ±8.63***	40.44 ±3.83	60.96 ±2.2***	33.86 ±5.47	31.46 ±1.78	31.87 ±2.96	23.59 ±2.96***	18.34 ±2.46	12.8 ±1.5**
A B50% hanol seed act(200)(G19)	55.74 ±5.22	65.89 ±5.5***	38.73 ±6.85	59.53 ±2.3***	32.92 ±2.65	30.98 ±2.58	35.57 ±2.88	27.86 ±1.86***	14.69 ±1.37	11.8 ±2.7***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
-P<0.01, *-P<0.001 when compared G2 vs G3, G16, G17, G18 and G19



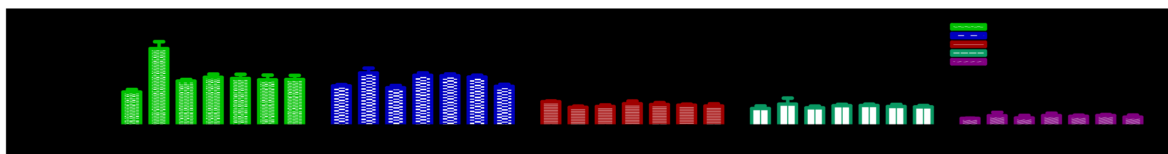
G1=Control, G2=Triton, G3=Atorvastatin, G16=AB aqueous seed extract(100), G17=AB aqueous seed extract(200), G18= AB 50% ethanol seed extract(100), G19= AB 50% ethanol seed extract(200)

Diet induced hyperlipidemia

Table 12: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	26.36 ±1.57###	22.30 ±3.87	35.87 ±8.36 ###	7.56 ±2.97	20.5 ±4.7###
Atorvastatin (G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.2***	31.13 ±3.6	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30 **	7.99 ±1.98	11.4 ±3.5***
AA aqueous leaf extract (100)(G4)	64.76 ±3.1	68.63 ±4.32***	55.34 ±1.43	71.37 ±3.3	34.86 ±1.23	31.38 ±3.65*	24.72 ±1.69	28.63 ±1.92 *	12.34 ±1.94	14.7 ±3.3*
AA aqueous leaf extract (200)(G5)	53.76 ±4.86	66.89 ±5.76***	44.65 ±3.39	70.75 ±2.5	33.34 ±2.34	30.25 ±2.55	21.94 ±1.86	28.89 ±1.76 *	9.65 ±1.39	13.75 ±1.5**
AA 50% ethanol leaf extract (100)(G6)	57.87 ±5.5	65.46 ±6.6***	39.54 ±3.54	68.86 ±2.54	33.24 ±1.76	29.39 ±1.32	27.87 ±2.15	27.46 ±2.86 *	9.54 ±2.32	14.8 ±1.2*
AA 50% ethanol leaf extract (200)(G7)	60.76 ±7.52	64.96 ±5.7***	38.66 ±5.85	55.86 ±2.73*** €€€	32.56 ±2.93	28.24 ±3.00	19.75 ±2.58	26.96 ±1.76 **	8.66 ±1.06	12.6 ±2.7***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2, €€€-P<0.001 when compared G6 vs G7, *-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G4, G5, G6 and G7

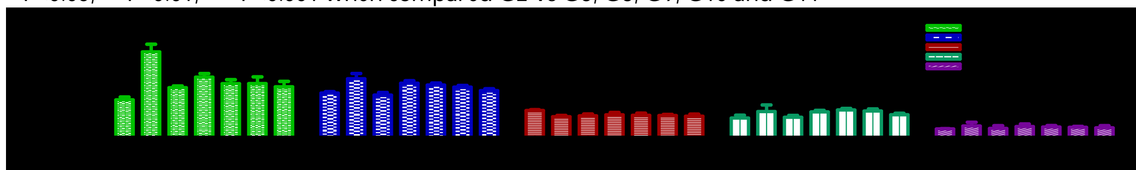


G1=Control, G2=Triton, G3=Atorvastatin, G4=AA aqueous leaf extract(100), G5=AA aqueous leaf extract(200), G6= AA 50% ethanol leaf extract(100), G7= AA 50% ethanol leaf extract(200)

Table 13: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	26.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	12.56 ±2.97	14.5 ±4.7###
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	7.99 ±1.98	11.4 ±3.1***
AA aqueous seed Extract (100)(G8)	62.75 ±2.89	76.68 ± 4.53***	58.53 ±1.46	68.93 ±3.1	44.78 ±1.53	28.69 ±2.97	26.67 ±1.57	32.47 ± 1.96	11.25 ±1.84	13.8 ±3.2**
AA aqueous seed Extract (200)(G9)	61.67 ±4.86	68.45 ±4.96***	46.79 ±3.34	66.68** ±2.5	38.46 ±2.33	28.15 ±2.63	22.26 ±1.97	34.99 ±1.83	10.43 ±1.29	13.2 ±1.8**
AA 50% ethanol seed Extract (100)(G10)	58.43 ±5.5	68.48 ±8.6***	43.44 ±3.83	63.96 ±2.2***	35.76 ±5.74	27.88 ±1.35	22.87 ±2.86	33.59 ±2.64	9.34 ±2.3	12.8 ±1.2***
AA 50% ethanol seed extract(200)(G11)	56.74 ±6.22	64.46 ±6.82***	45.73 ±6.85	59.53 ±2.3***	33.92 ±2.71	26.96 ±2.56	18.57 ±2.88	28.86 ±1.86*	8.69 ±1.3	12.2 ±2.7***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G8, G9, G10 and G11

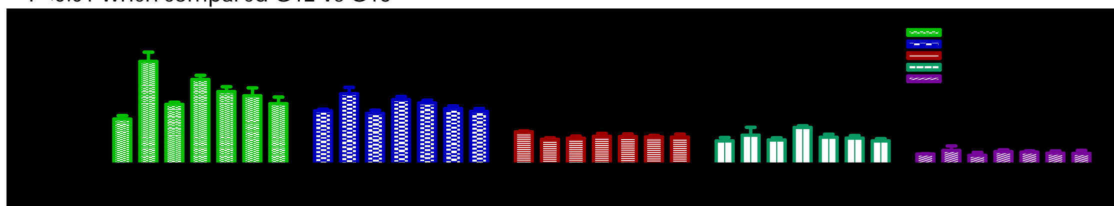


G1=Control, G2=Triton, G3=Atorvastatin, G8=AA aqueous seed extract(100), G9=AA aqueous seed extract(200), G10= AA 50% ethanol seed extract(100), G11= AA 50% ethanol seed extract(200)

Table 14: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	26.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	7.56 ±2.97	14.5 ±4.7###
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	27.53 ±2.14	15.92 ±0.95	25.64 ±2.30**	4.99 ±1.98	9.4 ±3.1***
AB aqueous leaf extract(100)(G12)	61.76 ±2.15	89.63 ±4.3***	42.34 ±2.13	68.37 ±3.2***	33.86 ±1.86	29.66 ±2.96	20.76 ±1.86	38.89 ±1.76	6.68 ±1.35	13.4 ±1.8**
AB aqueous leaf extract(200)(G13)	59.76 ±5.01	76.89 ±5.04***	40.65 ±3.17	64.75 ±2.9***	38.67 ±2.39	29.25 ±2.68	18.84 ±2.23	28.56 ±3.08*††	5.89 ±1.85	12.8 ±1.2***
AB 50% ethanol leaf extract(100)(G14)	56.87 ±5.5	72.46 ±8.4***	38.54 ±3.95	58.86 ±2.7***	33.63 ±1.76	28.88 ±1.56	18.57 ±1.67	27.57 ±2.65*	5.75 ±1.23	11.7 ±2.3***
AB 50% ethanol leaf extract(200)(G15)	54.76 ±6.22	64.06 ±6.94***	36.66 ±5.05	55.86 ±3.0***	34.9 ±2.6	28.76 ±2.95	16.92 ±0.95	24.64 ±2.30***	5.21 ±1.98	11.4 ±3.1***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
*-P<0.05, **-P<0.01, ***-P<0.001 when compared G2 vs G3, G12, G13, G14 and G15
††-P<0.01 when compared G12 vs G13

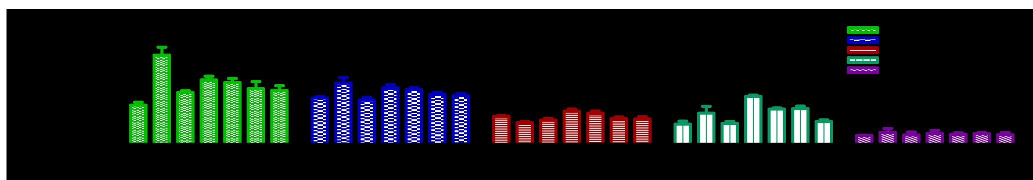


G1=Control, G2=Triton, G3=Atorvastatin, G12=AB aqueous leaf extract(100), G13=AB aqueous leaf extract(200), G14= AB 50% ethanol leaf extract(100), G15= AB 50% ethanol leaf extract(200)

Table 15: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in triton induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 h	24h	0 h	24h	0 h	24h	0 h	24h	0 h	24h
Control(G1)	47.8 ±3.6	47.8 ±3.6	56.4 ±1.78	56.4 ±1.7	34.2 ±1.04	34.2 ±1.04	20.65 ±3.54	20.65 ±3.54	10.8 ±0.35	10.8 ±0.3
Triton(G2)	62.54 ±4.59	108.82 ±9.70###	44.53 ±2.16	74.62 ±6.7###	35.4 ±2.32	28.36 ±1.57###	22.30 ±3.87	35.87 ±8.36###	26.56 ±2.97	14.5 ±4.7###
Atorvastatin(G3)	53.52 ±4.4	63.24 ±2.30***	35.76 ±4.7	53.86 ±3.1***	31.13 ±3.65	29.53 ±2.14	15.92 ±0.95	25.64 ±2.30***	7.99 ±1.98	11.4 ±3.1***
AB aqueous seed extract(100)(G16)	58.72 ±1.45	78.64 ±4.57***	42.53 ±1.46	68.93 ±3.7	34.78 ±1.8	40.46 ±2.52	28.67 ±1.45	28.47 ±1.48**	23.25 ±1.2	13.2 ±3.7**
AB aqueous seed extract(200)(G17)	58.67 ±3.86	75.35 ±4.96***	40.79 ±3.34	66.68 ±2.5**	34.46 ±2.33	37.86 ±2.55	18.26 ±1.43	22.99 ±1.24***	21.43 ±1.48	12.2 ±1.8***
AB 50% ethanol seed extract(100)(G18)	57.43 ±5.5	67.84 ±8.63***	40.44 ±3.83	60.96 ±2.2***	33.86 ±5.47	31.46 ±1.78	31.87 ±2.96	23.59 ±2.96***	18.34 ±2.46	12.8 ±1.5**
A B50% ethanol extract(200)(G19)	55.74 ±5.22	65.89 ±5.5***	38.73 ±6.85	59.53 ±2.3***	32.92 ±2.65	30.98 ±2.58	35.57 ±2.88	27.86 ±1.86***	14.69 ±1.37	11.8 ±2.7***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
-P<0.01, *-P<0.001 when compared G2 vs G3, G16, G17, G18 and G19



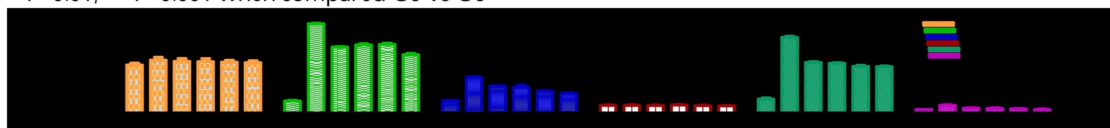
G1=Control, G2=Triton, G3=Atorvastatin, G16=AB aqueous seed extract(100), G17=AB aqueous seed extract(200), G18= AB 50% ethanol seed extract(100), G19= AB 50% ethanol seed extract(200)

Diet induced hyperlipidemia

Table 16: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.7	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.35	15.8 ±0.3
Atherogenic diet (G2)	156.67 ±6.87	268.63 ±14.7[#]	85.37 ±1.43	455.7 ±2.4[###]	55.97 ±1.86	180.8 ±2.96[###]	37.86 ±1.86	36.82 ±2.75[###]	139.52 ±4.13	386.21 ±5.3[###]	11.86 ±1.86	36.65 ±2.75[###]
AA aqueous leaf extract(100)(G3)	155.35 ±8.65	262.63 ±14.2	75.33 ±2.43	347.1 ±5.6***	59.55 ±1.24	135.2 ±1.24***	34.62 ±6.76	36.36 ±1.57	135.55 ±1.2	256.53 ±3.0***	10.87 ±15.6	22.36 ±1.57***
AA aqueous leaf extract (200)(G4)	154.86 ±7.86	260.87 ±15.6	74.87 ±2.1	336.3 ±1.3*** ☨☨	58.67 ±1.45	128.25 ±12.3***	35.37 ±3.25	38.66 ±2.96	134.67 ±1.45	252.76 ±4.7***	11.78 ±9.56	20.98 ±2.96***
AA 50% ethanol leaf extract (100)(G5)	153.24 ±7.67	258.78 ±9.56	74.35 ±7.34	349.3 ±6.5***	56.57 ±2.88	109.6 ±1.37***	34.34 ±9.59	35.45 ±2.16	134.57 ±2.88	236.88 ±5.04***	11.34 ±9.59	18.45 ±2.16***
AA 50% ethanol leaf extract (200)(G6)	156.48 ±5.5	255.46 ±9.56	70.87 ±4.3	296.7 ±6.5*** €€€	56.20 ±3.86	96.79 ±3.34*** €€	32.76 ±8.18	34.37 ±1.43	133.67 ±3.86	234.89 ±5.8***	13.97 ±1.07	15.35 ±2.96***

Values are expressed as mean±S.D; n=6.[#]-P<0.05,[###]-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G3, G4, G5 and G6
[€€]-P<0.01, [€€€]-P<0.001 when compared G5 vs G6

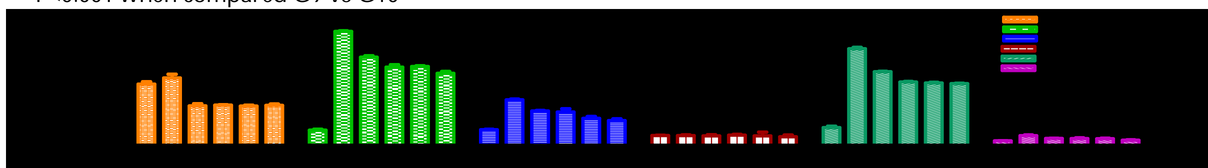


G1= Normal diet, G2= Atherogenic diet, G3=AA aqueous leaf extract(100), G4=AA aqueous leaf extract(200), G5= AA 50% ethanol leaf extract(100), G6= AA 50% ethanol leaf extract(200)

Table 17: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes aspera* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.5	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.35	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7###	85.37 ±1.43	455.7 ±2.4###	55.97 ±1.86	180.8 ±2.96###	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.2 ±5.3###	11.86 ±1.86	36.65 ±2.75###
AA aqueous seed Extract (100)(G7)	156.35 ±8.65	253.86 ±14.1***	81.06 ±1.2	352.3 ±5.3***	51.30 ±5.54	135.8 ±1.8***	31.59 ±2.96	35.86 ±4.54	70.57 ±2.88	293.7 ±1.36***	10.37 ±5.34	25.53 ±0.51***
AA aqueous seed extract (200)(G8)	159.55 ±1.24	255.25 ±1.24***	85.56 ±12.3	312.0 ±9.7*** †††	48.28 ±4.22	131.6 ±12.8***	31.86 ±1.86	38.65 ±2.75	68.92 ±0.95	252.5 ±1.9***	11.09 ±9.79	24.84 ±2.76***
AA 50% ethanol seed extract (100)(G9)	155.65 ±3.65	256.76 ±3.85***	75.12 ±8.71	314.8 ±2.7***	56.67 ±6.8	106.3 ±7.34***	32.43 ±3.67	35.86 ±14.84	64.57 ±2.88	248.3 ±2.4***	9.45 ±5.43	24.45 ±2.16***
AA 50% ethanol seed Extract (200)(G10)	158.45 ±5.37	252.43 ±3.67***	72.54 ±5.32	287.3 ±7.3*** €€€	53.35 ±8.65	97.30 ±6.7***	31.47 ±8.65	32.30 ±6.76	72.67 ±3.86	245.9 ±1.3***	13.84 ±2.1	18.37 ±1.43*** €€€

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G7, G8, G9 and G10 ,
†††-P<0.001 when compared G7 vs G8
€€€-P<0.001 when compared G9 vs G10

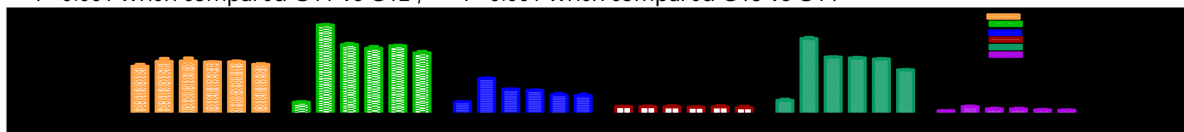


G1= Normal diet, G2= Atherogenic diet, G7=AA aqueous seed extract (100), G8=AA aqueous seed extract (200), G9= AA 50% ethanol seed extract(100), G10= AA 50% ethanol seed extract(200)

Table 18: Effect of aqueous and 50% ethanol leaf extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.3	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7##	85.37 ±1.43	455.7 ±2.2###	55.97 ±1.83	180.8 ±2.96###	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.2 ±5.3###	11.86 ±1.8	36.65 ±2.75###
AB aqueous leaf Extract(100)(G11)	166.35 ±8.65	270.86 ±14.8	81.06 ±12.9	355.3 ±5.6***	56.30 ±5.54	125.8 ±1.2***	31.59 ±2.96	35.86 ±4.5	138.5 ±2.88	290.7 ±1.3***	11.37 ±5.3	25.16 ±2.54***
AB aqueous leaf extract (200)(G12)	159.55 ±1.24	265.85 ±1.24	80.56 ±12.3	336.0 ±9.1*** †††	55.28 ±4.22	119.6 ±2.4***	28.86 ±1.86	33.65 ±2.75	132.9 ±0.95	286.4 ±1.9***	12.21 ±9.7	24.84 ±2.65***
AB 50% ethanol leaf extract (100)(G13)	163.65 ±3.65	266.76 ±3.85	75.12 ±8.76	348.8 ±2.7***	63.67 ±6.87	96.35 ±7.34***	29.43 ±3.6	35.86 ±4.84	127.5 ±2.82	281.1 ±2.4***	12.45 ±5.4	19.67 ±2.16***
AB 50% ethanol leaf extract (200)(G14)	168.45 ±5.3	253.43 ±3.67	65.54 ±5.32	312.3 ±7.3*** €€€	59.35 ±8.65	92.76 ±6.76***	27.47 ±8.65	31.30 ±6.76	118.6 ±3.86	225.3 ±1.6*** €€€	11.04 ±2.4	18.37 ±1.43***

Values are expressed as mean±S.D; n=6.##-P<0.01,###-P<0.001 when compared G1 vs G2
***-P<0.001 when compared G2 vs G11, G12, G13 and G14
†††-P<0.001 when compared G11 vs G12 , €€€-P<0.001 when compared G13 vs G14

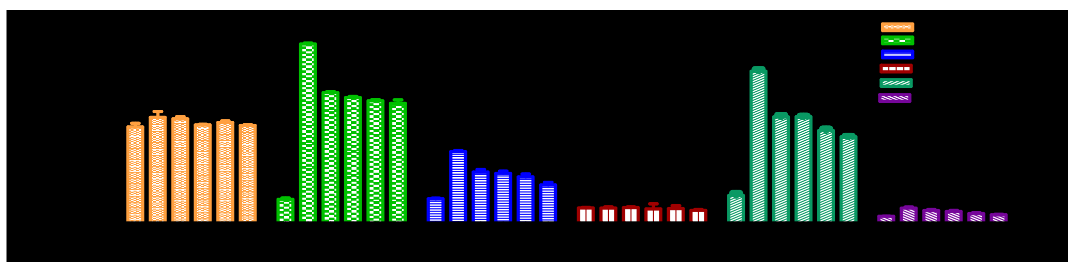


G1= Normal diet, G2= Atherogenic diet, G11=AB aqueous leaf extract(100), G12=AB aqueous leaf extract(200), G13= AB 50% ethanol leaf extract(100), G14= AB 50% ethanol leaf extract(200)

Table 19: Effect of aqueous and 50% ethanol seed extracts of *Achyranthes bidentata* on body weight, cholesterol, triglycerides, HDL, LDL and VLDL cholesterol in high fat diet induced hyperlipidemic rats

Treatment and dose (mg/kg b.w)	Body weight		Cholesterol		Triglycerides		HDL		LDL		VLDL	
	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week	0 week	4 week
Normal diet(G1)	154.43 ±3.54	243.75 ±9.57	47.8 ±3.6	58.8 ±3.6	56.4 ±1.78	60.4 ±1.73	34.2 ±1.04	37.2 ±1.04	60.80 ±3.54	68.80 ±3.54	10.8 ±0.3	15.8 ±0.3
Atherogenic diet(G2)	156.67 ±6.87	268.63 ±14.7###	85.37 ±1.43	455.7 ±2.4###	55.97 ±1.86	180.8 ±2.96###	37.86 ±1.86	36.82 ±2.75	139.5 ±4.13	386.8 ±5.3###	11.6 ±1.8	36.65 ±2.75###
AB aqueous seed extract(100)(G15)	168.37 ±10.6	264.66 ±5.5	80.86 ±14.8	331.5 ±2.9***	55.30 ±5.54	128.7 ±6.5***	34.21 ±3.33	37.86 ±1.86	135.5 ±2.88	269.5 ±4.8***	10.7 ±3.2	30.16 ±2.54***
AB aqueous seed extract (200)(G16)	165.86 ±1.34	248.86 ±1.84**	79.82 ±2.9	318.8 ±3.1***	52.28 ±4.22	125.3 ±5.72***	36.23 ±1.86	34.65 ±12.87	133.9 ±2.9	269.8 ±2.5***	11.4 ±5.7	27.84 ±2.65***
AB 50% ethanol seed extract (100)(G17)	169.76 ±7.18	255.43 ±3.67	74.46 ±1.18	310.4 ±3.5***	55.67 ±6.72	116.3 ±7.34***	35.43 ±3.67	35.35 ±7.34	125.5 ±3.54	234.5 ±4.8***	11.7 ±9.7	22.67 ±2.16***
AB 50% ethanol seed extract (200)(G18)	165.77 ±8.36	247.86 ±1.7	75.37 ±8.34	304.4 ±8.6***	52.30 ±5.5	95.76 ±6.76***	35.02 ±8.63	30.86 ±1.86	126.6 ±3.86	218.7 ±2.3*** €€€	12.8 ±5.3	19.54 ±1.43***

Values are expressed as mean±S.D; n=6.###-P<0.001 when compared G1 vs G2
-P<0.01, *-P<0.001 when compared G2 vs G15, G16, G17 and G18
€€€-P<0.001 when compared G17 vs G18



G1= Normal diet, G2= Atherogenic diet, G15=AB aqueous seed extract(100), G16=AB aqueous seed extract(200), G17= AB 50% ethanol seed extract(100), G18= AB 50% ethanol seed extract(200)

Histopathology studies

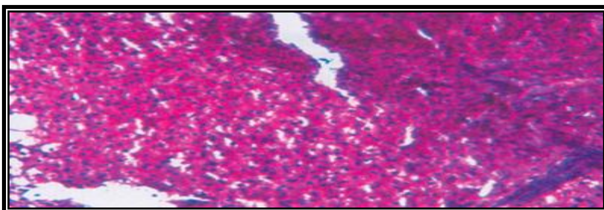


Figure 4: Section of the aorta from hyperlipidemic rat with numerous foam cells in media (high fat diet)

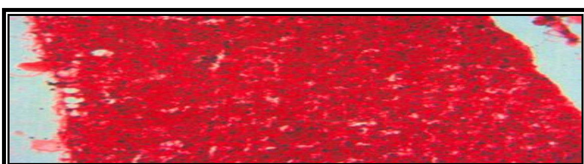


Figure 5: Section of the aorta showed no foam cells; only edema in the media. (50% ethanol leaf extract of *Achyranthes aspera*, 200 mg/kg b.w)

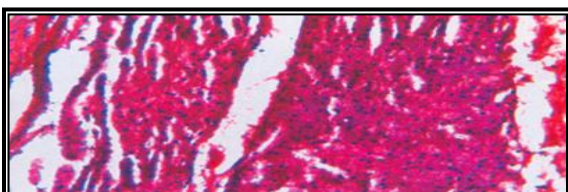


Figure 6: Section of the aorta showed no foam cells in the media (50% ethanol seed extract of *Achyranthes aspera*, 200 mg/kg b.w)

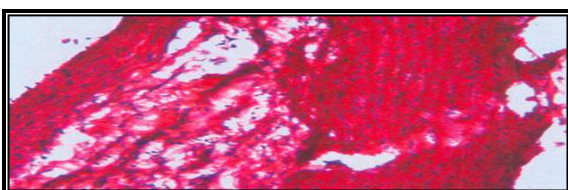


Figure 7: Section of the aorta showed no foam cells in the media (50% ethanol leaf extract of *Achyranthes bidentata*, 200 mg/kg b.w)

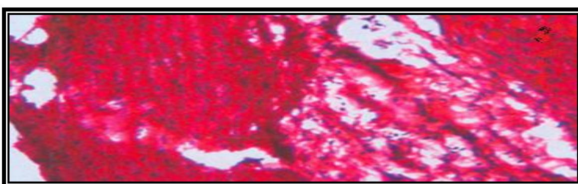


Figure 8: Section of the aorta showed no foam cells in the media (50% ethanol seed extract of *Achyranthes bidentata*, 200 mg/kg b.w)

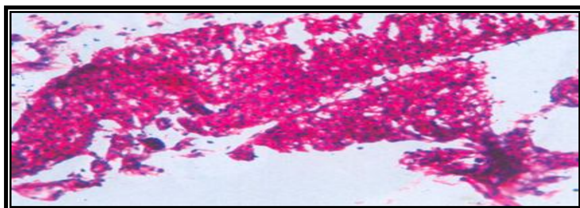


Figure 9: Section of the aorta showed few foam cells in the media (50% ethanol leaf extract of *Achyranthes bidentata*, 100 mg/kg b.w)

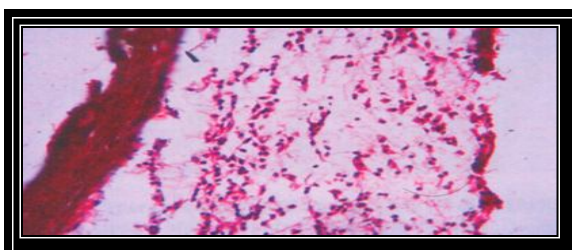


Figure 10: Section of the aorta showed few foam cells in the media (50% ethanol leaf extract of *Achyranthes aspera*, 100 mg/kg b.w)

DISCUSSION

The current anti-hyperlipidemic therapy includes principally statins and fibrates; the former corrects the altered blood lipid profile by inhibiting the biosynthesis of cholesterol and the latter acts by enhancing the clearance of triglyceride rich lipoproteins. The investigation on plant drugs will be a useful strategy in the discovery of new lead molecules eliciting improved activity by regulating the different mechanisms maintaining the lipid metabolism and thus can be used in treating hyperlipidemia of varied etiology [5]. *Achyranthus aspera* is already proved for its spermicidal, antilithiatic, antifertility, immunostimulatory, cancer chemopreventive activity [6, 7, 8, 9,]. *Achyranthus bidentata* is already proved for its cancer chemoprotective, osteoprotective, neuroprotective, and immunostimulatory [10]. But these plant species were also demonstrated to reduce the serum cholesterol levels. The anti-hyperlipidemic activity of *A.aspera* and *A.bidentata* had not been elucidated in an controlled hyperlipidemic animal models, the present study has been designed to evaluate the lipid – controlling activity of *A.aspera* and *A.bidentata* against triton-induced hyperlipidemia and high fat diet-induced hyperlipidemia, in male Wistar albino rats. On the induction with triton, hypercholesterolemia occurs through the mobilization of cholesterol from the liver into plasma compartment initially (first phase) and that subsequent to such mobilization increased synthesis of hepatic cholesterol occurs (second phase) which is accompanied by the elevation of 3-hydrocy -3-methylglutatyl-CoA reductase activity [11]. Intra peritoneal administration of resulted in an enormous elevation of serum cholesterol, triglycerides, LDL, and lowering of VLDL cholesterol levels at 24 hrs and the observations are on par with the previous studies .

Treatment with aqueous and 50% ethanol extracts of AA leaf and AA seed at two dose levels 100 mg/kg and 200 mg/kg showed significant activity in lowering the levels of cholesterol and VLDL in triton induced hyperlipidemic rats. The 50% ethanol extract of AA leaf and AA seed at a dose level of 200 mg/kg produced better activity in reducing the levels of triglycerides, LDL and VLDL showing the significant dose dependent activity. Regression of these markers is strongly supporting the antihyperlipidemic activity of 50% ethanol extract of AA leaf and AA seed (200 mg/kg).Aqueous and 50% ethanol extracts of AB leaf and AB seed at two dose levels 100 mg/kg and 200 mg/kg significantly lowered the levels of cholesterol,

triglycerides, LDL, and VLDL. Further, 200 mg/kg of both aqueous and 50% ethanol extract of AB leaf and AB seed produced a better activity when compared to lower dose showing the significant dose dependent activity. The results revealing that the AB leaf and seed extract possessed the better activity than AA leaf and seed extracts showing that the *A.bidentata* is having high potential in lowering the hypercholesterolemia. These results may indicate that the leaf and seed extracts of *A.aspera* and *A.bidentata* may interfere with cholesterol-biosynthesis as Triton accelerated the hepatic synthesis of cholesterol [12]. A high-cholesterol diet regarded as an important factor in the development of cardiac diseases, since it leads to the development of hyperlipidemia, atherosclerosis, and ischemic heart disease. A variety of mechanisms, i.e. inhibition of mevalonate pathway, decrease in NO bioavailability and cGMP metabolism, increase in free radical and peroxynitrite formation, inhibition of heat shock response, and expression of oxidized low density lipoprotein receptors which induces apoptosis, have been shown to play a role in cardiac effects of hyperlipidemia [13].

Triton induced hypercholesterolemia, though simple and rapid for evaluating hypolipidemic compounds, is rather artificial. Hence the lipid controlling potential of *A.aspera* and *A.bidentata* leaves and seeds were further evaluated in high-fat diet induced hyperlipidemic rat model. When male Wistar albino rats were kept on cholesterol-rich diet supplemented for 4 weeks and body weight, serum cholesterol, trigylcerides, LDL, VLDL levels were elevated where as HDL levels were significantly reduced. Elevated circulating lipid levels may be outcome of inhibitory effect of high fat dietary intake on lipogenesis. The treatment of high-fat diet induced hyperlipidemic rats with aqueous and 50% ethanol extracts of AA leaf and AA seed at two dose levels 100 mg/kg and 200 mg/kg showed significant activity in lowering the levels of cholesterol and VLDL. Though the levels of HDL were not increased, they are maintained as that of control rats. The extracts also possessed the significant activity in lowering the body weights. Further, the higher dose of aqueous and 50% ethanol extract of AA leaf and AA seed produced better activity in reducing the levels of cholesterol, triglycerides, and VLDL showing the significant dose dependent activity. Aqueous and 50% ethanol extracts of AB leaf and AB seed at two dose levels 100 mg/kg and 200 mg/kg significantly lowered the levels of cholesterol, triglycerides, LDL, and VLDL. The levels of HDL were not reduced and body weights are not increased, they are maintained as that of control rats. Further, the higher dose of aqueous and 50% ethanol extract of AB leaf and AB seed produced better activity in reducing the levels of cholesterol and LDL showing the significant dose dependent activity. The presence of foam cells resulted due to the proliferation of stromal cells is a characteristic feature of lipid deposition and atherosclerosis. Histopathology of aorta of rats treated with atherogenic diet shows presence of numerous foam cells which confirmed the induction of hyperlipidemia. Groups treated with 50% ethanol extract of leaf and seed of *A. aspera* and *A. bidentata* at a dose of 200 mg/kg showed absence of foam cells. Whereas few foam cells were found in case of groups treated with 100 mg/kg of 50% ethanol extract of leaf and seed of *A. aspera* and *A. bidentata*, supporting the dose dependent activity.

Presence of saponins, flavonoids, and triterpenoids in *A. aspera* and *A. bidentata* was already reported. Saponins derived from the *Acorus calamus* were reported to reduce the serum cholesterol levels by preventing the cholesterol absorption, interfering with entero-hepatic circulation and increasing its fecal excretion [14]. Flavonoids present in the *Pterocapus marsupium* were observed to be responsible for lowering the serum cholesterol, LDL and VLDL cholesterol levels . Lupeol, a triterpenoid was reported for its cardioprotective activity against experimental hypercholesterolemia . The lipid lowering activity of *A. aspera* and *A. bidentata* leaf and seed extract may be attributed to the presence of phytoconstituents such as saponins, flavonoids, and triterpenoids as reported for other plant extracts. Recent studies have demonstrated that increased formation of free radicals or reactive oxygen species (ROS) contribute to cardiovascular disease progression [15]. The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification and DNA breaks. ROS induced depletion of antioxidants is a key factor for initiation of atherosclerosis . The anti hyperlipidemic activity may be attributed to the inhibition of ROS by the

flavonoids and triterpenoids present in the extracts. All the beneficial effects of the extracts may be due to their antihyperlipidemic effects carried out by saponins, flavonoids, and triterpenoids present in them.

CONCLUSION

Achyranthes aspera Linn and *Achyranthes bidentata* Blume are two perennial herbs with slender and rambling branches. The two plant materials were collected from different parts of Nilgiri district of Tamilnadu and authenticated. Physicochemical parameters like total ash, acid insoluble ash, water soluble ash, and sulphated ash values were determined. Fluorescence analysis was carried out for the plant powder and their extracts.The dried and powdered leaves and seeds of plants were extracted with water and 50% ethanol through cold maceration. Alcohol soluble extractive and water soluble extractive values were determined. The phytochemical studies of the plant extracts showed the presence of alkaloids, glycosides, triterpenoids, saponins, flavonoids and mucilage. These results gave clues regarding the presence of some particular phytoconstituents in the respective plant extracts. The *in vivo* screening for antihyperlipidemic activity with triton induced hyperlipidemia and high fat diet induced hyperlipidemia model showed that both aqueous and 50% ethanol extracts of plants at a dose of 200 mg/kg exhibited significant activity against hyperlipidemia. Antihyperlipidemic activity activity of the various extracts may be attributed to the presence of triterpenoids, saponins, and flavonoids. From these studies, it can be concluded that both the plants are endowed with significant antihyperlipidemic activity. However, the future studies need to be carried out to isolate and screen the phytoprinciples responsible for the anti-hyperlipidemic activity.

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Indian Journal Of Natural Sciences *ISSN: 0976 - 0997*

Vol 1 / Issue 3 / December 2010 *© IJONS*

RESEARCH ARTICLE

Phytochemical Screening and Antibacterial Activity of Plant Extract of *Eclipta prostata* L.

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Received: 20 Oct 2010 Revised: 15 Nov 2010 Accepted: 1 Dec 2010

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ABSTRACT

Eclipta prostata.L (Asteraceae) is an important medicinal plant with lot of phytochemical constituents selected form the available literature was taken for the present study. The aqueous and ethanol extracts of *Eclipta prostata.L* were used to determine the antibacterial efficiency against Gram positive and Gram negative organisms such as *E.coli*, *Staphylococcus edudemis*, *Serratia marcenses* and *Salmonella typhimurium*. The ethanol extract of *Eclipta prostata.L* showed a strong inhibitory activity against all the four species of Gram positive and Gram negative organisms, while the aqueous extract of the plant had not produce inhibitory against any of the species used in the present study. From the result it could be concluded that the ethanol extract of *Eclipta prostata.L* as a strong antibacterial agent against both Gram positive and Gram negative organisms namely *E.coli*, *Salmonella typhimurium*, *Serratia marcenses* and *Staphylococcus edudemis*. Phytochemical screening revealed that plant extracts of *Eclipta prostata.L* contained flavonoids, saponins, phytosterols, carbohydrates, phenols, tannins and terpenoids.

Key words: *Eclipta prostate* L., Antibacterial activity, Phytochemical Analysis.

INTRODUCTION

Eclipta prostata.L (Asteracae) commonly known with the name of "Vellai karisinakanni" in Tamil nadu, India. Leaf juice is used as tonic for jaundice and leaf paste is applied on the affected area for tooth ache [1]. The roots are very rich of thiophene acetylenes. Four compounds were isolated from *E. prostrata*, of which

two were identified as stigmasterol and alpha-terthenyl. Alpha-terthienyl is isolated from plant and these constituents are significant for hepatoprotective activity [2, 3]. *Eclipta prostata.L* an aromatic plant is known in Chinese herbal medicine for the treatment of various kidney diseases. *Eclipta prostata.L* can play an important role in osteoblastic bone formation, and may possibly lead to the development of bone-forming drugs [4]. Leaf extract of *E. Prostrata* are shown Hypolipidemic activity [5].Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents, according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids, tannins and so on. Plants do not produce enough secondary compounds to protect all their tissues at all times, thus concentration and types of compounds vary through out the plant. Some compounds are present at all times in cellular tissue and some are activated after attack. The secondary metabolite production of desirable medical compounds from plants, bio-technological approach, especially plants tissue cultures are found to have potential as found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites [6]. The main aim of the work is to develop the appropriate extraction methods in order to obtain plant extracts with as many phytochemical compounds as possible is important.

MATERIAL AND METHODS

Plant extraction

Eclipta prostata.L were shade-dried and pulverized to a coarse powder. Equal quantities of the powder was passed through 40-mesh sieve and exhaustively extracted with 90% (v/v) ethanol in soxhlet apparatus at 60°C [7]. The extract was evaporated under pressure until all the solvent had been removed and further removal of the water was carried out by freeze drying to give an extract sample with the yield of 19.7% (w/w.) The extract was stored in refrigerator, weighed amount was dissolved in 2% (v/v) aqueous Tween–80 (2 ml of Tween 80 dissolved in 98 ml of water) and used for present investigation.

Preliminary phytochemical analysis

The Phytochemical screening procedures were carried out according to the following methods. By using the standard procedures to identify the constituents as described by Evans [8] and Harborne [9].

Test organisms

Both Gram positive and Gram negative organisms were used for the test. The Gram positive bacteria include *Staphylococcus edudemi* and *Serratia marcenses.* The Gram negative bacteria include *E.coli* and *Salmonella typhimurium.* All the bacterial strains were maintained on freshly prepared Muller and Hinton Agar (Hi-Media) slant and stored at 0°C.

Screening of antibacterial activity

Antibacterial activity was determined by agar well diffusion method. Muller Hinton agar plates were prepared after that the inoculums were swab with the help of cotton swab. The sterile well cutter was to cut 3 wells in each plate. The three crude solvent extract were poured on to the separate well. The plant extract was slowly diffused thorough media in the plates. The inoculated plates were incubated at 37°C for 24 hours.

RESULT AND DISCUSSION

Preliminary phytochemical Analysis
A Phytochemical are important component which was the agents causes the antibacterial activity. The amount of phytochemical may vary in different plants. The determination study of the phytochemical on *Eclipta prostata.L* gives these results (Table-1).

Thin layer chromatography was used for determination of important components of the plant extracts. The Rf value helps to find out the quantity of the detected objects and the colour helps to find out the object. The Rf Value is distance moved by the sample in Cm/Distance the moved by solvent in cm (Table-2). Phytochemically, *Eclipta prostata.L is* rich in wadeoloctone, eclalbasaponin, ß-amyrin, stigmasterol and luteolin-7- glucoside [10]. We have found that most of the biologically active phytochemicals were present in the ethanol extract of *Eclipta prostata.L*. The medicinal properties of extracts may be due to the presence of above mentioned phytochemicals.

The antibacterial activity of ethanol extracts against four microbial species was shown in Table-3. *Serratia marcenses, Salmonella typhimurium, Escheria coli, and Staphylococcus edidemis* are the micro organisms used. No inhibition was observed by the aqueous extract of *Eclipta prostata.L* against any of the microorganisms used in the present study. On contrast, the ethanol extract of *Eclipta prostata.L* was found to produce inhibition against the growth of all the four microorganisms used in the present study. The ethanol extracts of *Eclipta prostata.L* were found to be most effective inhibitor of bacterial growth of *Escheria coli*, *Salmonella typhimurium, Serratia marcenses* and *Staphylococcus ebidemis*. In general *S.marcescens* is not pathogenic to insects when present in the digestive tract in small numbers, but once it enters the hemocoel it multiples rapidly and cause death in one to three days. The inhibitory zone size of gram positive bacteria indicates their less sensitive when compared to the Gram negative bacteria. It may be due to the presence of large amount of peptidoglygon layer which protect against various compounds [11]. The result obtained from this study is compliment to earlier observations by Rotimi [12]. The result of the present study confirms the antimicrobial potential of the ethanolic extracts of *Eclipta prostata.L.*

CONCLUSION

The results of the present *in vitro* study indicated that the ethanol fraction of *Eclipta prostata.L* leaf extract possess effective antibacterial properties which is as potent as standard antibacterial properties against certain microorganisms.

ACKNOWLEDGEMENT

Authors are thankful to J.J College of arts and science for providing the facilities for me. I would thank to Mr.Joshva, Miss. Jebitha, Miss. Latha, for encouraging me to do my project. I would like to thank I.Raja, R.Palathasar and A.Jude for providing fund to me carrying out my project.

Table: 1. Preliminary phytochemicals analysis of *Eclipta prostata.L*

Phytochemicals	Inference	%
Flavonoids	+	0.045
Saponins	+	0.029
Phytosterols	+	0.026
Carbohydrates	+	0.016
Phenols	+	0.019
Tannins	+	0.009
Terpenoids	+	0.004
Alkaloids	-	-
Phlobatannis	-	-

+ = Presence; **-** = Absence

Table: 2. Thin Layer chromatographic determinations in *Eclipta prostata.L*

Colour	Rf Value
Pink(Amino acids)	0.1
Green(Neutral lipids)	0.13
Brown(Mono-Disaccharides)	0.36

Table: 3. Antibacterial activities of the various extracts of *Eclipta prostata.L*

Name of the microorganism	Plant Extract	Solvent	Diameter of inhibition of Zone (mm)	
			Control	Test
E.coli	*Eclipta prostata.L*	Water	-	-
		Ethanol	-	11mm
S.typhimurium	*Eclipta prostata.L*	Water	-	-
		Ethanol	-	8mm
S.marcenses	*Eclipta prostata.L*	Water	-	-
		Ethanol	-	7mm
S.ebidemis	*Eclipta prostata.L*	Water	-	-
		Ethanol	-	5mm

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