Anthelmintic and Antioxidant efficacy of two Macrolichens of Ramalinaceae

S.V Praveen Kumar[1], T.R Prashith Kekuda[2], K.S Vinayaka[3], S.J Sudharshan[2]

[1] P.G Dept. of Studies and Research in Microbiology, Shivagangothri, Tholahunase, Davangere, Karnataka, INDIA
[2] Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, INDIA
[3] P.G. Dept. of Studies and Research in Applied Botany, Jnanasahyadri, Shankaraghatta-577451, Karnataka, INDIA

ABSTRACT

Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. In this study, the anthelmintic and antioxidant efficacy of methanol extract of two macrolichens of Bhadra wildlife sanctuary namely *Ramalina hossei* H. Magn & G. Awasthi and *Ramalina conduplicans* Vain. were investigated. Extracts of both lichens exhibited concentration dependent anthelmintic activity as revealed by paralysis and death of adult Indian earth worms. Among lichen extracts, *R. hossei* showed potent anthelmintic activity as compared to *R. conduplicans*. The DPPH free radical scavening and Fe^{+3}reducing assay showed potent antioxidant activity of extracts in a dose dependant manner. *R. hossei* exhibited high free radical scavenging activity than *R. conduplicans*. Metabolites namely usnic acid, sekikaic acid, selanizic acid, tannins and others were detected in methanol extracts of the lichens. The results of the present study suggest that the lichen extracts could be used as natural antioxidants and could be used to control helminthic infections. Further studies are to be taken to isolate active constituents and determine the antioxidant and anthelmintic efficacy *in vitro* and *in vivo*.

Keywords: Macrolichens, Bhadra wildlife sanctuary, Ramalina hossei, Ramalina conduplicans, Antioxidant activity, Anthelmintic activity
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** ks.vinayaka@rediffmail.com

INTRODUCTION

Helminthic infections are among the most common infections in man, affecting a large proportion of the world's population. Today, the principal mode for control of gastrointestinal parasites is based on the commercial anthelmintics. Because of the increasing anthelmintic resistance and the impact of conventional anthelmintics on the environment, it is important to look for alternative strategies against gastrointestinal nematodes. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. It is in the context that people consume several plants or plant derived preparations to cure helminthic infections (1). Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (2). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (3, 4). In recent years much attention has been devoted to natural antioxidant and their association with health benefits (2). India is a rich center of lichen diversity, contributing nearly 15% of the 13,500 species of lichens so far recorded in the world (5). Lichens and lichen products have been used in traditional medicines for centuries and still hold considerable interest as alternative treatments in various parts of the world. They produce characteristic secondary metabolites that are unique with respect to those of higher plants (6). In various systems of traditional medicine worldwide, including the Indian system of medicine, these lichen species are said to effectively cure dyspepsia, bleeding piles, bronchitis, scabies, stomach disorders, and many disorders of blood and heart (7-9). Bhadra reserve area (75°15'-75°50' E and 13°25'-13°50' N latitude)

comprises the forests of Western Ghats and its fringes. Sanctuary being situated in the south interior Karnataka, with cool climate throughout the year and affords pleasant days during the hot months. *Ramalina hossei* H. Magn & G. Awasthi (Ramalinaceae) is a tufted, errect, fruticose lichen with thallus corticolous, tufted, errect, yellowish grey in colour, branched. *Ramalina conduplicans* Vain. (Ramalinaceae) is a fruticose lichen with thallus corticolous 3–5 cm long, decumbent, greenish grey colour and branched (10). The present study was conducted to evaluate the anthelmintic and antioxidant efficacy of above two macrolichens of Bhadra wildlife sanctuary.

MATERIALS AND METHODS

Collection and Identification of lichens

The lichens namely *R. hossei* (Voucher no. KSV/KU00905) and *R. conduplicans* (Voucher no. KSV/KU101073) growing on trees in sanctuary were collected and identified by morphological, anatomical, chemical tests (10). Secondary metabolites were identified by Thin layer chromatography (TLC) in solvent A (180 toluene: 60 1-4, dioxine: 8 acetic acid) (11, 12). The lichen specimens were preserved in Department of Botany for future reference.

Extraction of powdered lichen material

For extraction, 20 g portions of each powdered lichen material was taken and added to 100 ml of methanol. The mixture was sonicated for 30 min, and then left at room temperature overnight. The extract was filtered over Whatman No 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass (13). The extracts were tested for the presence of alkaloids, tannins, steroids, saponins, flavonoids and terpenoids (14). The condensed methanol extracts were used determining biological activities.

Anthelmintic activity of methanol extracts

The assay was performed on adult Indian earthworm *Pheretima pasthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Standard drug (Piperazine citrate, 5%) and methanol extracts (5, 10 and 20mg/ml) were prepared in 0.85% normal saline and poured into respective labeled petriplates (50 ml). A saline control was kept. Six worms of nearly equal size were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors (15). Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased (1).

Antioxidant activity of lichen extracts
DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed to determine the antioxidant activity of lichen extracts and the standard Ascorbic acid (16, 17). The methanol extracts (0.25, 0.50 and 1.00mg/ml) and DPPH (0.002%) were prepared in methanol. Equal volume of different concentrations of methanol extracts and DPPH were mixed in clean and labeled test tubes separately and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation.

$$\text{Scavenging activity (\%)} = A - B / A \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

Fe^{+3} reducing power assay

Different concentrations of Methanolic extracts (0.25, 0.50 and 1.00mg/ml) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50°C, cooled rapidly, mixed with 2.5ml of 10% trichloroacetic acid and 0.5ml of 0.1% Ferric chloride. The intensity of iron (II)-ferricyanide complex was determined by measuring the formation of Perl's Prussian blue at 700nm after 10min. The higher absorbance of the reaction mixture indicates increased reducing power (16, 18).

RESULTS AND DISCUSSION

TLC in solvent A showed the presence of Usnic acid and Sekikaic acid in *R. hossei*. Metabolite namely Selanizic acid was detected in *R. conduplicans* in addition to Usnic acid and Sekikaic acid. Preliminary phytochemical analysis of methanolic extracts showed the presence of tannins in both the lichen extracts. In addition to tannins, terpenoids were detected in *R. hossei* and steroids were detected in *R. conduplicans* (Table-1).

In this study, extracts of both *R. hossei* and *R. conduplicans* exhibited marked anthelmintic activity in

Table 1: Chemical constituents detected in TLC and Phytochemical screening of extracts of lichen species

Metabolite	R. hossei	R. conduplicans
Usnic acid	+	+
Selanizic acid	–	+
Sekikaic acid	+	+
Alkaloids	–	–
Terpenoids	+	–
Tannins	+	+
Saponins	–	–
Flavonoids	–	–
Steroids	–	+

'+' Detected; '–' Not detected

terms of causing paralysis and death of worms (Table-2). The paralysis time and death time in 5% piperazine citrate was found to be 16 and 28 minutes respectively. The extracts exhibited dose dependent activity. Among lichen extracts, *R. hossei* showed potent anthelmintic activity by causing paralysis and death of worms in shorter time as compared to *R. conduplicans*.

The result of antioxidant activity of methanol extracts of lichen species by DPPH free radical scavenging assay is shown in Table-3. The crude solvent extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extracts have exhibited concentration dependent radical scavenging activity i.e., higher the concentration, more scavenging potential. Among the lichen extracts, *R. hossei* exhibited high free radical scavenging activity as compared to *R. conduplicans*. Neither extracts showed activity as potent as the standard (ascorbic acid). The result of reducing power of methanol extracts of selected lichen species and tannic acid is represented in Table-4. In this study, the absorbance was found to increase with the dose of methanolic extracts and standard which is suggestive of reducing power.

Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycobacterial, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects. Even though these manifold activities of lichen metabolites have now been recognized, their therapeutic potential has not yet been fully explored and thus remains pharmaceutically

Table 2: Anthelmintic activity of different concentrations of methanol extracts

Concentration (mg/ml)	Paralysis time (in min)		Death time (in min)	
	R. hossei	*R. conduplicans*	*R. hossei*	*R. conduplicans*
5	63	96	102	126
10	51	79	61	89
20	20	25	35	38

Table 3: Antioxidant potential of different concentrations of methanol extracts and standard by DPPH assay

Concentration (mg/ml)	Scavenging activity in %		
	R. hossei	*R. conduplicans*	Ascorbic acid
0.250	56.11	48.04	92.52
0.500	61.53	59.01	95.12
1.000	79.05	72.63	97.33

Table 4: Antioxidant potential of different concentrations of methanol extracts and standard by by Fe^{+3} reducing power assay

Concentration (mg/ml)	Absorbance at 700nm		
	R. hossei	*R. conduplicans*	Tannic acid
0.250	0.196	0.188	0.331
0.500	0.310	0.293	0.469
1.000	0.625	0.601	1.045

unexploited (19). The utility of lichens is due of range of secondary compounds produced by them. A wide range of secondary metabolites of lichens were characterized. According to their chemical structure, most lichen substances are phenolic compounds, dibenzofuranes, Usnic acids, depsidones, depsones, lactones, quinines and pulvunic acid derivatives (20). In this study, secondary metabolites such as usnic acid, sekikaic acid, and Salazinic acid are reported to be present in the lichen extracts.

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. Parasitoses have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances (21). The results of this study have shown promising anthelmintic activity suggesting the possible use of lichens extracts in intestinal nematode control. The anthelmintic activity of methanol extracts could be due to the constituents present.

There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (22). The antioxidant activity of lichen extracts might be due to the presence of various groups of phytochemicals in them. In this study, the scavenging activity was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. For the measurements of reductive ability, we have investigated the Fe^{3+} reducing power in the presence of methanol extracts of selected formulations. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity (23). An increase in the absorbance revealed the reducing power of extracts. In this study, the reducing power of methanolic extracts was found to increase with the dose. The antioxidant activities have been reported to be the concomitant development of reducing power (24).

The traditional medicines hold a great promise as a source of easily available effective anthelmintic and antioxidant agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of natural for their biological efficacy. However, their scientific evaluation as compared to commercial agents is limited. Helminthic worms constitute a major public health menace and have been positively related with various types of illness. Nowadays even there is risk of development of resistance by these parasites to the drugs. In light of this, the results of the present study suggest that the lichen extracts could be used in the control of helminthic infections namely Ascariasis etc as the worms used in the study are in resemblance with the intestinal parasitic worms such as *Ascaris lumbricoides*. The extracts have also showed promising results in terms of their antioxidant potential and thus could be exploited as natural antioxidants.

ACKNOWLEDGEMENT

The authors express their sincere thanks to HOD, Department of Microbiology and Principal, S.R.N.M.N College of Applied Sciences, Shimoga. Authors also thank N.E.S for providing all facilities and moral support to conduct work.

REFERENCES

1. Temjenmongla and Yadav A.K., Anticestodal efficacy of folklore medicinal plants of Naga tribes in Northeast India. *Afr. J. Trad. CAM*. **2**: 129–133 (2005).
2. Ali S.S., Kasoju N., Luthra A., Singh A., Sharanabasava H., Sahu A. and Bora U., Indian medicinal herbs as sources of Antioxidants. *Food Research International*. **41**: 1–15 (2008).
3. Kumpulainen J.T. and Salonen J.T., Natural Antioxidants and Anticarcinogens in Nutrition, *Health and Disease*. The Royal Society of Chemistry, UK. 178–187 (1999).
4. Cook N. and Samman S., Flavonoids- Chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry*. **7**: 66–76 (1996).
5. Negi H. R., On the patterns of abundance and diversity of macrolichens of Chopta-Tunganath in the Garhwal Himalaya. *J Biosci*. **25**: 367–378 (2000).
6. Lawrey J.D., Biological role of lichen substances. *Bryologist*. **89**: 111–122 (1986).
7. Saklani A. and Upreti D.K., Folk uses of some lichens in Sikkim. *J Ethnopharmacol* **37**: 229–233 (1992).

8. Lal B. and Upreti D.K., Ethnobotanical notes on three Indian lichens. *Lichenologist*. **27**: 77–79. (1995).
9. Negi H.R. and Kareem A., Lichens: The unsung heroes. *Amrut*. **1**: 3–6 (1996).
10. Awasthi D.D., *A Compendium of the Macrolichens from India, Nepal and Sri Lanka*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India. 2000.
11. Culberson C.F., Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *Journal of Chromatography* **72**: 113–125. (1972).
12. Walker F.J. and James P.W., A revised guide to microchemical technique for the identification of lichen products. *Bull. Brit. Lich. Soc*. **46**: 13–29 (1980) (Supplement).
13. Yilmaz M., Turk A.O., Tay T. and Kivanc M., The antimicrobial activity of extract of the lichen *Cladonia foliaceaandits* (-) Usnic acid, atranorin and fumarprotocetraic acid constituents. *Z. Naturforsch*. **59c**: 249–254 (2004).
14. Parekh J., Chanda S.V., In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turk J.Biol*. **31**: 53–58 (2007).
15. Grime A.S., Bhalke R.D., Ghogare P.B., Tambe V.D., Jadhav R.S. and Nirmal S.A. Comparative in vitro anthelmintic activity of *Mentha piperita* and *Lantana camara* from Western India. *Dhaka Univ. J. Pharm. Sci*. **5**: 5–7 (2006).
17. Khalaf N.A., Shakya A.K., Al-Othman A., El-Agbar Z., and Farah H., Antioxidant activity of some common plants. *Turk.J.Biol*. **32**: 51–55 (2008).
16. Ravikumar Y.S., Mahadevan K.M., Kumaraswamy M.N., Vaidya V.P., Manjunatha H., Kumar V. and Satyanarayana N.D., Antioxidant, Cytotoxic and Genotoxic evaluation of Alcoholic extract of *Polyalthia cerasoides* (roxb) Bedd. *Environmental Toxicology and Pharmacology*. **26**: 142–146 (2008).
18. Oyaizu M., Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.*, **44**: 307–315 (1986).
19. Muller K., Pharmaceutically relevant metabolites from lichens. *Applied Microbiology and Biotechnology*. **56**: 9–16 (2002).
20. Boustie J. and Grube M. Lichens as a promising source of bioactive secondary metabolites. *Plant Genetic Resources*. **3**: 273–287 (2005).
21. Nunomura R.C.S., daSilva E.C.C., Oliverira D.F., Garcia A.M., Boeloni J.N., Nunomura S.M., Pohlit A.M., *In vitro* studies of the anthelmintic activity of Picrolemma sprucei Hook.f. (Simaroubaceae). *Acta Amazonica*. **36**: 327–330 (2006).
22. Koleva I.I., Vanbreek T.A., Linssen J.P.H., Groot A.D.E. and Evstatieva L.N. Screening of plant extracts for antioxidant activity: A comparative study on the three testing methods. *Phytochem. Anal*. **13**: 8–17. (2002).
23. Meir S., Kanner J., Akiri B. and Hadas S.P., Determination and involovement of Aqeous reducing compounds in Oxdative Defense systems of various senescing Leaves. *Journal of Agricultural Food Chemistry*. **43**: 1813–1817 (1995).
24. Yang J.H., Lin H.C. and Mau J.L., Antioxidant properties of severea commercial Mushrooms. *Food Chemistry*. **77**: 229–235 (2002).

Anthelmintic Potential of *Andrographis paniculata, Cajanus cajan* and *Silybum marianum*

Singh Siddhartha*, Mehta Archana, John Jinu and Mehta Pradeep

Plant Biotechnology lab, Department of Botany, Faculty of Life Sciences,
Dr. H. S. Gour University, Sagar (M.P), India - 470 003

ABSTRACT

Hydroalcoholic extracts of aerial parts of *Andrographis paniculata, Cajanus cajan* and *Silybum marianum* and their combinations were evaluated for anthelmintic properties using Indian adult earthworms (*Pheretima posthuma*) as a model, as they show physiological and anatomical resemblance with intestinal parasites and round worms. The results were compared with that of standard drug, piperazine citrate. *A. paniculata* showed better activity (paralysis and death time was 3.33 and 5.16 min respectively at a concentration of 40mg/ml), while its combination (1:1) with *S. marianum* extract was found to be the most potent (paralysis and death time was 2.83 and 6.33 min respectively at a concentration of 40mg/ml). This property is supposed to be due to the presence of phenolics (flavonoids and tannins) which are reported to have anthelmintic property.

Keywords: Anthelmintic property, *Andrographis paniculata, Cajanus cajan*, earthworm model, *Silybum marianum*.

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** siddhartha.research@rediffmail.com

INTRODUCTION

Helmenthiasis is a highly prevalent disease mainly in the third world countries (1). Inadequate sanitation and poor hygiene may be the main reason behind the helmenthic infection (2). It is among the most important animal diseases inflicting heavy production losses. The gastro intestinal helmenthis has become resistant to the commonly used helmenthic drugs (3) and hence, there is a need to find the anthelmintic property from natural resources like medicinal plants, which may be effective against helmenthiasis and may have less or no side effects. *A. paniculata*, *C. cajan* and *S. marianum* belongs to family Acanthaceae, Compositae and Fabaceae respectively and shares some common medicinal properties such as antibacterial, antioxidant, hepatoprotective, etc (4-6).

MATERIALS AND METHODS

Plant material

The authenticated plants were collected from Natural Remedies Pvt. Ltd., Bangalore (sample invoice No. D119) and confirmed at Botany Department, Dr. H. S. Gour University, Sagar (M.P).

Chemicals and drugs

The following drugs and chemicals were used: Sodium chloride (HIMEDIA), Ethanol (RANKEM) and Piperazine citrate, a gift obtained from R. K. Pharmacy, Sagar.

Extract preparation

Dried and powdered plant materials were extracted with 70% ethanol using soxhlet apparatus. The extracts were concentrated and dried at 68°C and kept at 4°C for further studies.

Phytochemical test

Phytochemicals screening were performed to detect the presence or absences of various compounds such as tannins, flavonoids, alkaloids etc. as per standard methods (7).

Experimental model

The earthworms show physiological and anatomical resemblance with intestinal parasites and round worms (8) and were used for the anthelmintic assay. Indian adult earthworms (*Pheretima posthuma*, 5-7cm in length) were

freshly collected from organic farm of local earthworm breeding centre (N.H. 26, Sagar).

Experiment

The experiments were carried out by the method described by Jinu *et al*., 2009 (9). All the three extracts were dissolved in normal saline and diluted to get final concentrations of 20 and 40 mg/ml. Piperazine citrate (20 mg/ml) was used as a standard drug. Sixteen groups, each containing six earthworms were taken for the experiment. Extracts and piperazine citrate were dissolved separately in their respective concentration in 10ml normal saline. For combinational study, the different extracts were taken in the concentration ratio of 1:1. Time taken for paralysis and death of individual earthworm was noted. The paralysis occurs when the worms were placed in normal saline and were not able to move. The death was confirmed when the earthworms lost their motility and fading off their body colors (10).

RESULTS AND DISCUSSION

Biological activities of the plant extracts are due to the presence of various chemicals present in it. The activity of these phytochemicals depends upon the solvent used and the method of extraction (11). 70% ethanolic extracts showed maximum concentration of phenolic compounds (flavonoids). All the three plants analyzed for their anthelmintic potential showed a concentration depended activity. *A. paniculata* extract (40mg/ml) showed better activity (paralysis at 3.33 min and death at 5.16 min), while its combination with *S. marianum* extract (1:1) was found most potent (paralysis at 2.83 min and death at 6.33 min). The time taken by the standard drug (20mg/ml) for the paralysis and death of the worms was 7.0 and 14.83 min respectively. *C. cajan* extract showed the least activity, as it took longest time for paralysis (13.66 min) and death (17.0 min) of the worms (Table I).

Synthetic anthelmintic drugs like piperazine citrate, is known to cause paralysis of worms so that they are expelled in the feaces of men and animals. The extracts in the present study could cause paralysis as well as the death of the worms. These drugs may reach the target site in worms either orally or by diffusion and/or uptake through the cuticle, however the major uptake of the drug is through cuticle (12). All the three extracts when taken in combination, generally showed synergistically better activity as compared to individual effect. The variation in activity of the plant extract might be due to the difference in the proportion of the active compounds responsible for the anthelmintic property (13). The active

"Table I: Anthelmintic activity hydroalcoholic extracts of three plants"

Extract	Concentration (mg/ml)	Paralysis time (min.)	Death time (min.)
A.paniculata (Ap)	20	5.33 ± 0.40	7.50 ± 0.20
	40	3.33 ± 0.18	5.16 ± 0.14
C. cajan (Cc)	20	20 ± 0.23	25.83 ± 0.33
	40	13.66 ± 0.50	17 ± 0.23
S.marianum (Sm)	20	6 ± 0.23	10.16 ± 0.43
	40	3.83 ± 0.14	7.5 ± 0.31
Ap + Sm (1:1)	20	5 ± 0.23	8.5 ± 0.20
	40	2.83 ± 0.14	6.33 ± 0.18
Ap + Cc (1:1)	20	8.33 ± 0.18	12 ± 0.23
	40	5.5 ± 0.20	8.5 ± 0.31
Cc + Sm (1:1)	20	10.50 ± 0.20	13.66 ± 0.18
	40	6.33 ± 0.18	10 ± 0.23
Ap + Cc + Sm (1:1:1)	20	6.66 ± 0.38	10.50 ± 0.45
	40	4 ± 0.23	8.16 ± 0.36
Piperazine	20	7 ± 0.33	14.83 ± 0.36
Control (Normal saline)	-	-	-

All the values are expressed as mean ± SEM, N=6

constituents may be the phenolics such as flavonoids and tannins present in the plant extracts.

CONCLUSION

A. paniculata, C. cajan and S. marianum are well known for their medicinal properties (hepatoprotective and antioxidant). The present study on anthelmintic potential of these plants proves that their consumption may be good for health enhancement as well as intestinal problems.

ACKNOWLEDGEMENTS

The authors are grateful to Head, Department of Botany, Dr. H. S. Gour University, Sagar (M.P) for providing the facilities.

REFERENCES

1. Dhar D.N., Sharma R.L. and Bansal G.C. Gastrointestinal nematodes in sheep in Kashmir. *Vet. Parasitol*. **11**: 271–7 (1982).
2. Chan M.S. The global burden of intestinal nematode infections. *Fifty years on Parasitol Today*. **13**: 438–43 (1997).
3. Geert S. and Dorny P. Anthelmintic resistance in helminthes of animals of man in the tropics. Bulletin-des-Seances, *Academic Royale des Sciencesd. Dutre Mer.* **3**: 401–23 (1995).
4. Siddhartha K.M., Neelam S., Sangwan and Rajender S.S. *Andrographis paniculata* (Kalmegh): A Review. *Pharmacognosy Network Worldwide*. **1**: 283–298 (2007)
5. Wu N., Fu K., Fu Y.J., Zu Y.G., Chang F.R., Husan Y., Chen, Liu X.L, Kong Y., Liu W. and Gu C.B. Antioxidant Activities of extracts and Main Components of Pigeonpea [*Cajanus cajan* (L.) Millsp.] Leaves. *Molecules*. **14**: 1032–1043 (2009).
6. Bosisio E., Benelli C. and Pirola O. Effect of the flavanolignans of *Silybum marianum* L. on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes. *Pharmacol Res*. **25**: 147–154 (1992).
7. Harborne J.B. *Phytochemical methods*, 3rd edition, (Chapman & Hall, London, 1988) 117–119.
8. Nirmal S.A., Malwadkar G. and Laware R.B. Anthelmintic activity of *Pongamia glabra*. *Songklanakarin Journal of Science and Technology*. **29**(3): 755–757 (2007).
9. John J., Mehta A., Shukla S. and Mehta P. A report on anthelmintic activity of *Cassia tora* leaves. *Songklanakarin Journal of Science and Technology*. **31**(3): 269–271 (2009).
10. Girme A.S., Bhalke R.D., Ghogare P.B., Tambe V.D., Jadhav R.S. and Nirmal S.A. Comparative *In vitro* Anthelmintic Activity of *Mentha piperita* and *Lantana camara* from Western India. *Dhaka University Journal of Pharmaceutical Sciences*. **5**(1–2): 5–7 (2006).
11. Eloff J.N. Which extractant should be used for screening and isolation of antimicrobial components from plants? *J Ethnopharmacol*. **60**: 1–8 (1998).
12. Geary T.G, Sangster N.C. and Thompson D.P. Frontiers in anthelmintic pharmacology. *Vet Parasitol*. **84**: 275–295 (1999).
13. Eguale T., Thilahun G., Gidey M. and Mekonnen Y. *In vitro* anthelmintic activities of four Ethiopion medicinal plants against *Haemonchus contortus*. *Pharmacologyonldine*. **3**: 153–165 (2006).

The analytical and toxicological profiles of the red dye from the heart wood of *Caesalpinia sappan*

Divakar Madhu C.[1]*, Susheel John V.[2] and Lakshmidevi S.[3]

[1]Crescent college of Pharmaceutical sciences , Payangadi.P.O, Kannur, Kerala, India-670358,
[2]Sri Ramakrishna College of Pharmacy, SRIPMS, Coimbatore, Tamil Nadu, India, 641 044.
[3]Sri Ramakrishna College of Pharmacy, SRIPMS, Coimbatore, Tamil Nadu, India, 641 044.

ABSTRACT

Three phenolic red pigments were observed in combination from the heart wood of *Caesalpinia sappan* (F: Caesalpiniaceae). Preliminary cytotoxic and acute toxicity studies indicated that these natural colour pigments were safe compared to the FDC grade coal tar dyes. The isolated colour extract (CSWC) was highly soluble in water and analysed by spectrophotometric and HPTLC methods.

Keywords: *C. sappan* heart wood, red dye, toxicity, UV and HPTLC analysis.
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** madhu.divakar@gmail.com

INTRODUCTION

Caesalpinia sappan (F: Caesalpiniaceae) is a small spreading tree bearing orange red heart wood growing in South India, Srilanka and Malaysia. A decoction of the wood in water is used extensively for drinking purpose in Kerala. Literature survey (1, 2) indicated that the heart wood of *C.sappan* contains several constituents like amino acids, sugars, glycosides, steroids, flavonoids, saturated and unsaturated fatty acids, and phenolic compounds. The wood of *C.sappan* is also known as brazil wood, contains a red pigment called Brazilin (11) reported (3) to have anti-inflammatory activity, and also found useful in cosmetics. In the traditional system (4) of medicine the wood decoction is used for mild dysentery, diarrhoea, bronchitis, wounds, goitre, erysipelas, and also as an astringent & emmanogogue.

Nowadays, many different types of synthetic colours included in the class of coal tar dyes are used in pharmaceuticals and nutraceuticals. The production and processing of these synthetic dyes and colourants were estimated to release many hundred tonnes of unfixed and non-degradable waste which are hazardous to human health, and also might cause serious environmental pollution and ecological imbalance. The World Health Organisation (5) specified and set control limits for the use of these synthetic colours because of their toxic reactions towards eyes and skin.

In the light of the above findings an attempt has been made, to extract the colour pigments present in the heart wood of *C.sappan*, and to generate the analytical and toxicological profiles of these natural chromogens for use in industry as an alternative for the synthetic FDC colours.

EXPERIMENTAL

Plant material

C.sappan wood was collected in the month of March 2004 from North Parur, Cochin, Kerala and authenticated at the Department of Horticulture, The Tamil Nadu Agricultural university, Coimbatore, Tamil Nadu. The sample specimens (specimen no. PGSY/CCOPS/112) are preserved at the herbarium of the Dept. of Pharmacognosy, Crescent College of Pharmaceutical Sciences ,Payangadi, Kannur, Kerala -670358.

Extraction procedure

1kg of *C.sappan* wood was ground to a fine powder form and treated with petroleum ether and then soxhleted with distilled water for 2 hrs. The aqueous extract obtained

was filtered and concentrated *in vacuum* and kept in a micro wave oven for 5 min to make the dry powder (% yield 10.5).

HPTLC analysis of colour extracts CSWC

The HPTLC of the aqueous colour extract, (designated as CSWC: *C.sappan* wood colour) was done in a CAMAG WINCATS planar. Chromatography software using pre-prepared TLC plates (silica gel $60F_{254}$ MERK, Band width : 8mm, Slit dimension : 6x0.45mm, Wave length of scanning : 570nm) indicated the presence of three compounds (Rf value 0.91, 0.76, 0.61) using solvent system chloroform : methanol : water (64:50:10) and alcoholic ferric chloride as the detecting agent. The quantitative determination was done and the results are tabulated in table, Fig –3.

UV spectrophotometric analysis of the colour extracts CSWC

The colour extract CSWC was dissolved in double distilled water and scanned at 400-800nm in Jasco V-530 UV Spectrophotometer and obtained a λmax value of 538nm. But the λmax values for CSWC in pH:4 and in pH 10 were 443nm and 537nm respectively. A standard curve was plotted by taking various concentrations of CSWC (100–1000 mcg/ml) against absorbance at a wavelength maximum of 538nm. The results are plotted in Fig –1, 2(a), 2(b) and 4.

Preliminary Cytotoxicity Studies (8)

This study was conducted by Brine Shrimp Assay (BSA) method[8]. The brine shrimps eggs were hatched in a rectangular chamber and filled with artificial sea water and then ten numbers each of nauplii were transferred to vials using a pipette. The survival rate of the brine shrimps were observed after 24h for various concentrations of CSWC and a synthetic colour 'orange red' (Gold Camel FCF 15985, 14720, ISI no.5346). The LC_{50} values for these two colours were found out from the dose response graph. The results are tabulated in Fig –5.

Acute Toxicity Studies

The acute toxicity study (9, 10) was carried out using overnight fasted swiss albino mice. Both the colours CSWC and the synthetic colour (FCF.15985, 14720) were dissolved in distilled water and administered intra peritonially in doses of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g / kg body weight to different groups of mice of 6–10 each. Animals were observed at regular intervals of 1h for a period of 24h for death due to acute toxicity. Similarly the LD_{50} value was determined by giving CSWC and the FDF colour through oral route to respective group of animals

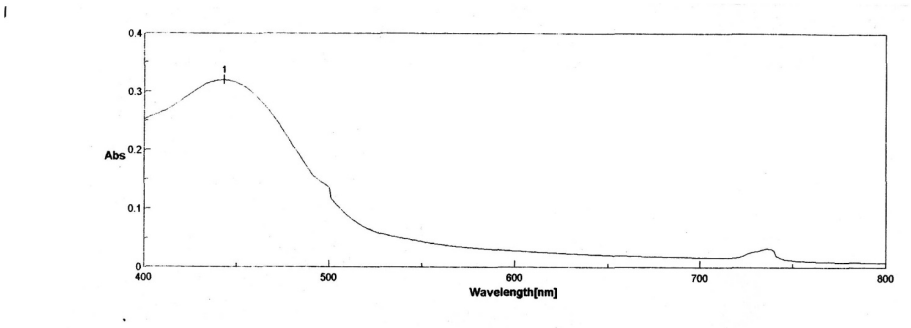


Figure 1- UV Spectrum of CSWC at pH4 (λ max 443nm)

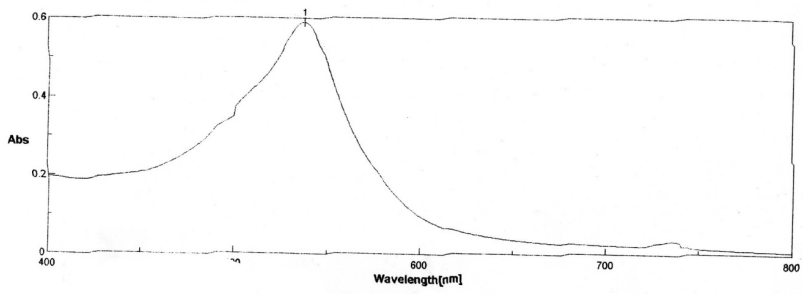


Figure 2(a)- UV Spectrum of CSWC at Normal pH (λ max 538nm)

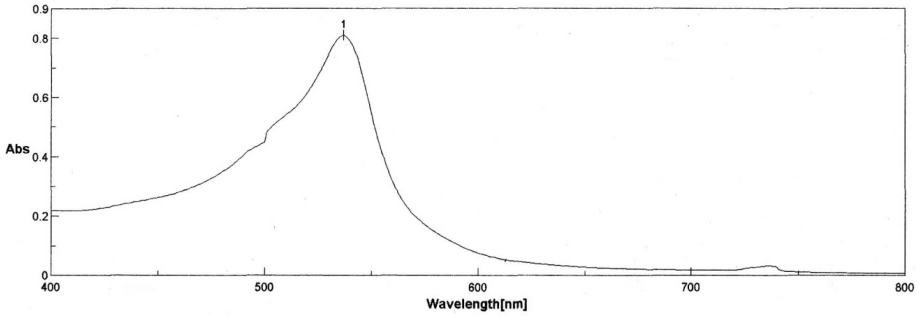
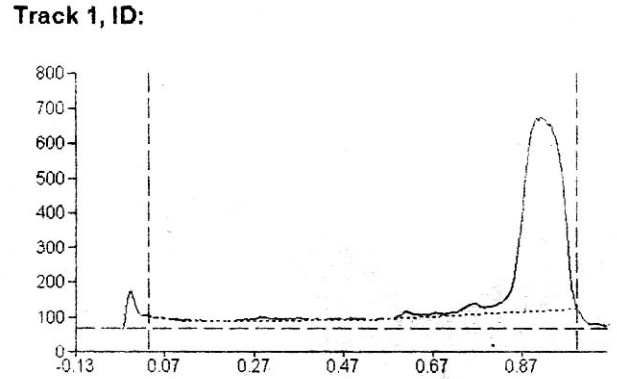


Figure 2(b)- UV Spectrum of CSWC at pH 10 (λ max 537nm)



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.58	1.3	0.61	19.1	3.14	0.63	8.8	443.9	1.03
2	0.72	9.6	0.76	32.3	5.29	0.79	19.1	1140.1	2.64
3	0.80	19.7	0.91	557.9	91.57	0.99	3.5	41624.3	96.33

Figure 3- High Performance Thin Layer Chromatogram of CSWC

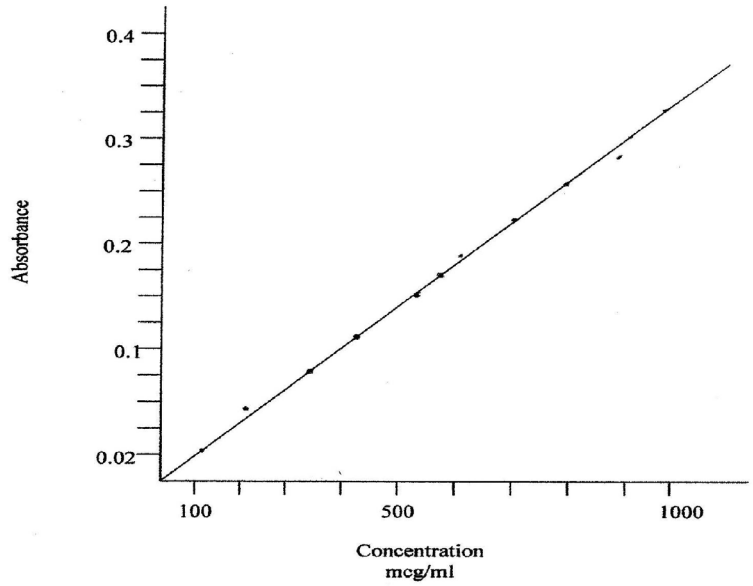


Figure 4- Standard curve of CSWC, concentration vs Absorbance

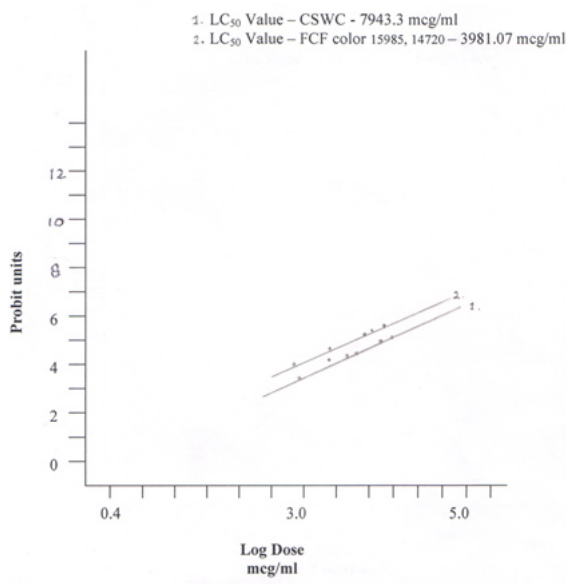


Figure 5- Dose Response curveLC_{50} determination(Brine Shrimp Assay Method)

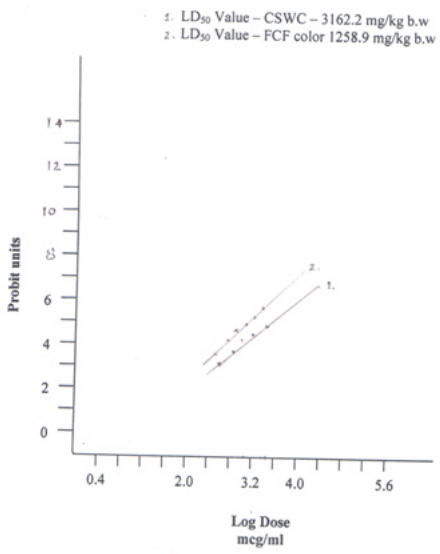


Figure 7- Dose Response curveRoute of Administratoion : Intra peritonial

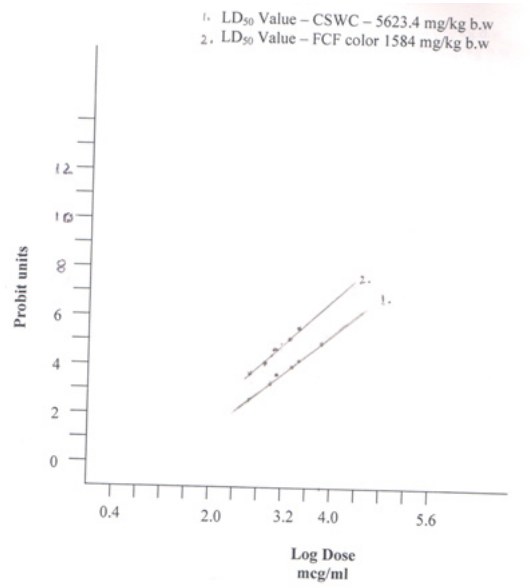


Figure 6- Dose Response curveRoute of Administration : Oral

by suspending the colours in 0.5% sodium carboxymethyl cellulose. The results are tabulated Fig –6 and 7.

All the animal experiments were conducted in accordance with National Institute of Health and Institutional Ethics Committee guidelines.

RESULTS AND DISCUSSION

The colour extract CSWC obtained from *C.sappan* wood was found to be highly soluble in water, therefore this colour can be incorporated easily in any pharmaceutical formulations like syrups, suspensions etc and also in food materials and nutraceuticals.

The UV spectral data indicated that CSWC at 100 µg/ml can give measurable absorbance at a λmax of 538nm in normal pH 7 and 443 nm in pH 4 and 537 nm in pH 10. The bright yellow and carmine red colour variation of CSWC in acidic and alkaline pH can be of use to prepare many shades of colours from this red pigment.

The colour extract CSWC and the synthetic red colour (FCF 15985, 14720) showed an LC_{50} value of 7943.3 and 3981.07 mcg/ml in the brine shrimp assay method. CSWC showed an LD_{50} value of 5623.4 mg/kg body weight and 3162.2 mg /kg b.w respectively in oral and intraperitonial routes of administration in acute toxicity studies. While the synthetic colour (FCF.15985, 14720) showed an LD_{50} value of 1584 mg/kg b.w and 1258.9 mg/kg b.w respectively in oral and intraperitonial routes of administration. These values indicated that the natural colour pigment CSWC is much safer compared to its synthetic counter part. HPTLC evaluation indicated the presence of three phenolic compounds in the aqueous extract CSWC with Rf values 0.91, 0.76 and 0.61 and quantified as 963, 26.4 and 10.3 mg/g of CSWC respectively. Reports (2, 4) indicated the anti-inflammatory, antiulcer and astringent properties of this natural colour pigments, therefore these herbal colours can be utilized in pharmaceuticals, cosmeceuticals and food materials not only as a colouring agent, but can impart many useful therapeutic activities also.

CONCLUSIONS

The results indicated that the colour extract CSWC obtained from *C.sappan* wood is very safe to use as a colouring agent in pharmaceutical and cosmeceuticals instead of synthetic FDC grade colours. The percentage yield (35.5%) of CSWC was found satisfactory for any commercial purposes. Also the results showed that the routine analysis and validation of CSWC can be done effectively by UV and HPTLC techniques.

ACKNOWLEDGEMENT

The authors were thankful to late Dr.S.B.Rao, Pharmaceutical consultant and former visiting Professor, Faculty of Medicine, University of Kerala for providing valuable references to carry out this research project.

REFERENCES:

1. Claus E P., Pharmacognosy, 3rd edn. (Lea and Febiger, Philadelphia), 1956, 210.
2. *The wealth of India*, *Raw materials*, Vol. **III**, (Publication and Information Directorate, CSIR, New Delhi), 1992, 14–16.
3. Hikino H., Anti-inflammatory activity of the red dye of *Caesalpinia sappan* heart wood, *Planta Med*, **31**, (1977), 214.
4. Chopra R N., Nayar S L and Chopra I C., "*Glossary of Indian Medicinal Plants*", (CSIR, New Delhi), 1956, 44.
5. WHO, *Quality control guidelines for Medicinal Plant Materials* (WHO Geneva) 1998, 1–11.
6. Stahl E., *Thin layer chromatography, A laboratory Hand book*, 2nd edn, (Springer International Student Edn., Berlin, New York), 1969, 705.
7. Farnsworth N R., Biological and Phytochemical screening of plants, *J. Pharm. Sci*., **55**, (1966), 225.
8. Meyer B N., Ferringni N R., Putnam J E., Jacobson LB., Nichols D E and Mc Laughlin J L., A Convenient General Bioassay for Active Plant Constituents, *Planta. Med*, **45**, (1982), 31.
9. Emmanuel B and Thomson A., Drug Bio screening, Fundamentals of Drug Evaluation Techniques in Pharmacology, *J.Pharm Sci*., **67**, (1978), 10.
10. Litchfield J T and Wilcoxon F J., A simplified method of evaluating dose effect experiments, *J. Pharm. Exp. Therap*., **96**, (1949), 99–113.
11. Harborne J B and Baxter H., *Phytochemical dictionary, A Hand Book of Bioactive compounds from plants* (Taylor and Francis), 1995, 542

Preliminary Evaluation of *Cassia auriculata* Seed Mucilage as Binding Agent

Singh S.K.*[1], Ushir Y.V.[2], Chidrawar R.V.[3], Vadalia K.R.[4], Sheth N.R.[5] and Singh S.[6]

[1, 2, 3, 4,] *Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot, (Guj), India*
[5]*Dept. of Pharmaceutical sciences, Saurasrta University, Rajkot, (Guj), India*
[6]*C.P.S. Mahuda college of Pharmaceutical sciences, Bhermpur, (Oris), India*

ABSTRACT

Cassia auriculata is a tropical tree locally belonging to the family Caesalpiniaceae found widely in India. Seeds of plant contain gluco-mannose hence attempt to evaluate the seeds for suitability as tablet binder is considered and the present investigation reports the isolation of mucilage of *Cassia auriculata* seed. The DSC and FTIR thermograms of drug and gum indicated no chemical interaction. Phytochemical characteristics of mucilage, such as carbohydrate, protein and flavanoids etc. were studied. Physiochemical characteristics of mucilage, such as solubility, swelling index, loss on drying, viscosity, hydration capacity, powder porosity, microbiological properties and pH were studied. The mucilage was evaluated for its granulating and binding properties in compressed tablet, using Diclofenac sodium as model drug. Mucilage was used in four different concentration i.e. 0.25, 0.5, 0.75 and 1.0% w/v. The granules were prepared by wet granulation process. The prepared granules were evaluated for percentage of fines, average particle size, compressibility index and flow properties. The properties were compared with Guar gum, which was used as standard binder at 1.00% w/v concentration. The tablet were prepared and evaluated for content uniformity, hardness, friability, disintegration time and *in vitro* dissolution profile. The tablets had good physiochemical properties, and the drug release was more than 85% within 3 hour. It was observed that increasing the concentration of mucilage increases hardness and decreases the disintegration time. All the formulations (F1, F2, F3, F4 and F5) were subjected to stability studies for three months at 25°C/60% RH, 30°C/65% RH and 40°C/75% RH as per ICH guidelines, only F1 and F2 showed stability with respect to release pattern and other parameters which confirm the use of mucilage as excipient.

Keywords: *Cassia auriculata*, Mucilage, Hydrogel, Binder, Dicolfenac sodium.
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** +912813298393 ; +919978819269 ; sudarshansingh83@gmail.com

INTRODUCTION

The high cost of imitation polymer and ecological pollution by chemical diligence has made the scientist in budding country to enter into era, in which plant products serve as alternative to synthetic products because of local accessibility, environmental gracious nature, subordinate prices and nontoxic compared to imported synthetic products. Today we have number of plant based pharmaceutical excipients such as guar gum, starch, agar, alginate, acacia, cocoa butter, cellulose etc. These natural excipients are used as binder, disintegrants in tablet, protective colloids in suspensions, thickening agent in oral liquids, gelling agents in gels and based suppositories. Similarly many plants restrain mucilage, which provide high concentration of complex sugar and uronic acid unit.

Mucilage and gums have been known since ancient times for their medicinal uses. In the contemporary era also they are widely used in the pharmaceutical industries as thickeners, water retention agents, emulsifying agent, suspending agents, binders and film formers (1-2). Apart from its use in finished medicines, newer uses have been found in the preparation of cosmetics, textiles and paint paper, hence the demand for these substances is increasing and new sources are getting tapped (3-4). Though, India due to geographical and environmental positioning has traditionally been a good source for such products among the Asian countries, a large quantity of this is still being imported from the European countries to meet up the ever-increasing demand (5). Of all the orally administered dosage forms, tablet is most preferred because of ease of administration, compactness and flexibility in manufacturing. Because of changes in

various physiological functions associated with aging including difficulty in swallowing, administration of intact tablet may lead to poor patient compliance and ineffective therapy.

Cassia auriculata is available locally belonging to the family Caesalpiniaceae and has not been explored as pharmaceutical excipients. The roots and seeds are astringent, cooling, depurative, alexateric, leprosy, tumors, asthma, urethrorrhoea, leaves are used in ulcers. Flowers are used in diabetes. The seed of *Cassia auriculata* swells and form gelatinous mass when it comes in contact with water due to its hydrophilic nature. Hence the present work was attempted to evaluate binding properties of seed mucilage of *Cassia auriculata*.

MATERIAL AND METHOD

Cassia auriculata seeds were procured from the forest of KORBA, Chhattisgarh, India. Diclofenac sodium was obtained as gift sample from Active Pharmaceutical Ingredient. All other ingredient were of analytical grade and purchased from Loba Chemicals, Mumbai.

Isolation of Mucilage from Cassia auriculata Seeds

Cassia auriculata seeds Kernel's powder (20g) were soaked in cold distilled water (200 ml) and slurry was prepared. Then slurry was mixed with 800 ml of boil distilled water. The solution was boiled for 20 minutes under stirring condition in water bath. The resulting thin clean solution was kept overnight for settling protein and fibers. The solution is centrifuge at 5000 rpm for 20 minutes. The supernant was separated and poured in to twice the volume of absolute acetone by continues stirring to precipitate the polysaccharides. The precipitate was washed with absolute ethanol, diethyl ether and petroleum ether and then dried at 40–45°C and passed through sieve #120 and stored in desiccators until used for further studies (6–8).

Drug-Excipients Compatibility Studies

This study has been done to check whether there is any compatibility related problems are associated with drug and the excipients used for the formulation of tablet. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, and easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. Thermal analysis and FTIR can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

FTIR Spectroscopy

The IR spectral analysis of a drug and other excipients were taken using Press pellet technique (using KBr). The IR spectra's were determined by using 1601 PC Shimadzu UV Spectrophotometer (9-12).

Differential Scanning Calorimeter Studies (DSC)

DSC was performed on a Shimadzu DSC-60 (Shimadzu Limited Japan). A 1:1 ratio of drug and excipient was weighed into aluminum crucible and sample was analyzed by heating at a scanning rate of 10°C/min over a temperature range 20°300°C under a nitrogen flow of 40ml/min. Reproducibility was checked by running the sample in triplicate (13-14).

DSC was performed on a Shimadzu DSC-60 (Shimadzu Limited Japan). A 1:1 ratio of drug and excipient was weighed into aluminum crucible and sample was analyzed by heating at a scanning rate of 10°C/min over a temperature range 20°300°C under a nitrogen flow of 40ml/min. Reproducibility was checked by running the sample in triplicate (13-14).

Preliminary Phytochemical Screening of Isolated Mucilage

The phytochemical properties such as presence of carbohydrate, protein, flavanoids, sterols, alkaloids, tannins, saponins and terpenoids determined (15).

Physicochemical Properties of Isolated Mucilage

The physicochemical properties such as solubility, pH and viscosity of dried mucilage were determined at 20°C. The loss on drying (LOD), ash values (total ash content, acid insoluble ash and water soluble ash) were determined according to Ayurvedic Pharmacopoeia of India (A.P.I) (16).

Microbiological properties

Microbial Load

Preparation of Inoculums

1g powder of *Cassia auriculata* mucilage was suspended in 10 ml of sterile water (inoculum). 1ml of inoculum was transferred to 99ml dilution blank (sterile water) which was diluted inoculum.

Plate Count Technique

Inoculum (1 ml) and diluted inoculum (1 ml) were transferred to separate petridishes 9 to 10 cm in diameter. After addition of both the inoculum to the plate, 20 ml of agar medium (40-45°C) was poured in to a each plate. Both the plates were gently rotated for through distribution of inoculum throughout the medium and solidified (17–19).

Preparation and Evaluation of Granules

Diclofenac sodium was used as model drug to formulate the granules. Lactose and aerosil was used as diluents and lubricant respectively. Binder solution was prepared by dissolving the mucilage of the *Cassia auriculata* in water at 0.25%, 0.50%, 0.75% and 1.0% w/v concentrations. The batch size was 100gm. The drug, lactose and aerosil were mixed thoroughly and sufficient volume of 20 ml of 0.25%, 0.50%, 0.75% and 1.0% w/v mucilage of *Cassia auriculata* (MCA) was added slowly to powder blend, and kneading was performed for near about 10 min until the formation of wet mass with enough cohesiveness. The wet mass forced through the sieve # 18 and dried at 40-45°C in hot air oven for 40 min the dried granules were received through sieve # 20. The prepared granules were then evaluated for percentage of fines, particles size and flow properties by measurement of angle of repose (20–21). The bulk and tapped densities of the granules were then assessed in accordance with the USP XXV tapped volume meter apparatus compressibility index of the granules was determined by Carr's compressibility index (22–23).

Preparation and Evaluation of Tablet

Tablets were compressed by using single punch machine (Cadmach) using flat faced punches. Each tablet weighed 200mg (Table 1). The prepared tablets were evaluated for content uniformity, hardness, disintegration time and *in vitro* dissolution profile using method specified in Indian Pharmacopoeia (I.P) (17).

RESULT AND DISCUSSION

The dried and coarsely powdered seeds of *Cassia auriculata* yielded high percentage (14.6% w/v) of mucilage using acetone as mucilage precipitating solvent. The thermogarm of drug and mucilage shows that there is no change in melting point which confirms that there is neither change in crystallinty of the drug nor any interaction further it was confirmed by FTIR spectral analysis (Figure 1, 2). The phytochemical screening of natural mucilage confirmed polysaccharides in nature (Table 2). The physicochemical and microbiological properties of MCA were determined. The MCA completely soluble in warm water, swelling index, viscosity obtained 25% and 3.51cps. The pH of the mucilage was found to be 5.8 were very near to neutral it may be less irritating on gastrointestinal tract and hence gum is suitable for uncoated tablet (Table 3). The extracted and purified natural gum were evaluated for microbial load, MCA shows 120 CFU per gram of gum which shows mucilage were under microbial limit (Table 4). The prepared granules were evaluated for percentage of fines, flow Properties ((Table 5). It was observed that percentages of fines were reduced as the concentration of mucilage was increased. The percentage of fines was little higher in granules prepared using 0.25% of mucilage as binder. The flow properties of granules were determined by angle of repose which was found to be 28° to 32°. Hence all the granules exhibited good flow properties. Bulk densities of the prepared granules were found to decrease of prepared granules were found to decrease slightly by increasing the concentration of MCA. This result may be due to the formation of larger agglomerates and decrease in fines in the granules, as increasing MCA concentration. The result of compressibility index indicates decrease in flow ability with increasing MCA concentration. However, all formulation showed good flow properties. In general

Table 1: Composition of Tablet Formulation

Ingredient	Seed mucilage of *Cassia auriculata* as binder				Guar gum
	F1	*F2*	*F3*	*F4*	*F5*
Diclofenac sodium	50 mg	50 mg	50 mg	50 mg	50 mg
MCA	Q.S	Q.S	Q.S	Q.S	Q.S
Lactose	Q.S	Q.S	Q.S	Q.S	Q.S
Aerosil	4 mg	4 mg	4 mg	4 mg	4 mg
Total weight	200 mg	200 mg	200 mg	200 mg	200 mg

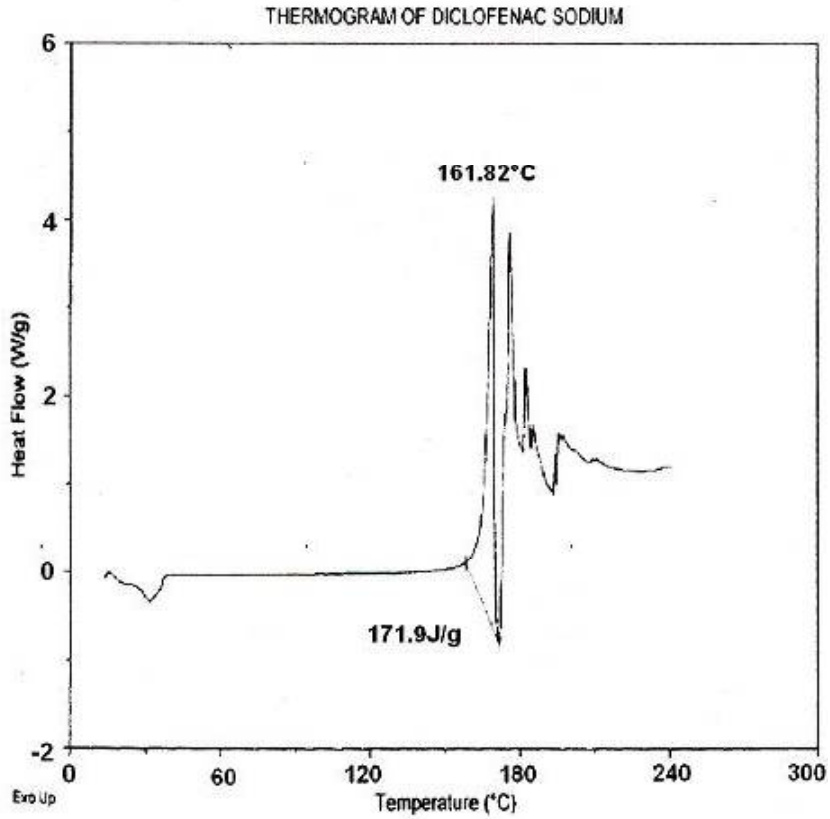


Figure 1: *DSC Thermogram of Diclofenac sodium*

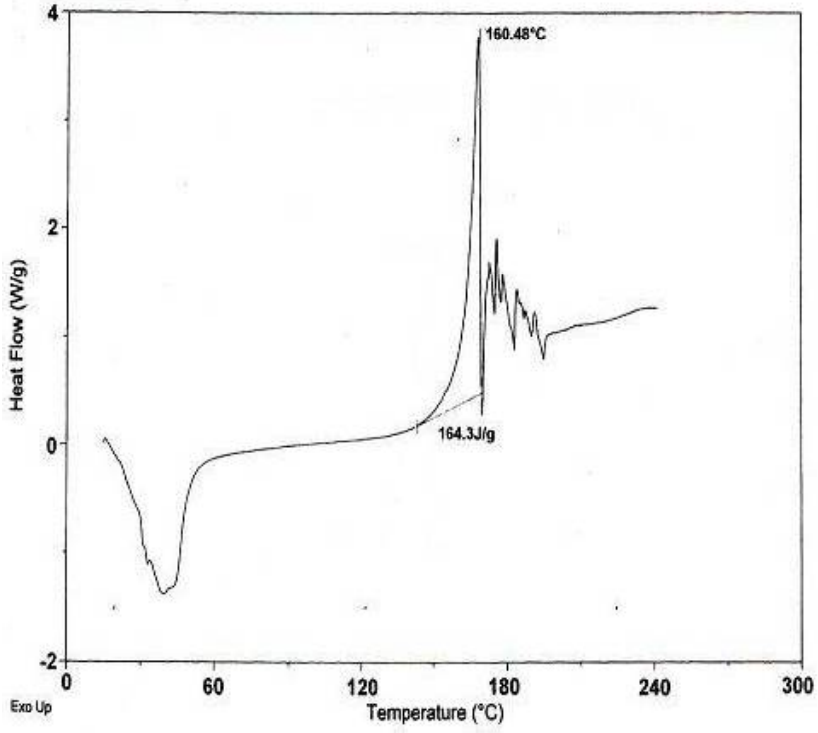


Figure 2: *DSC Thermogram of Diclofenac Sodium and MCA Physical Mixture*

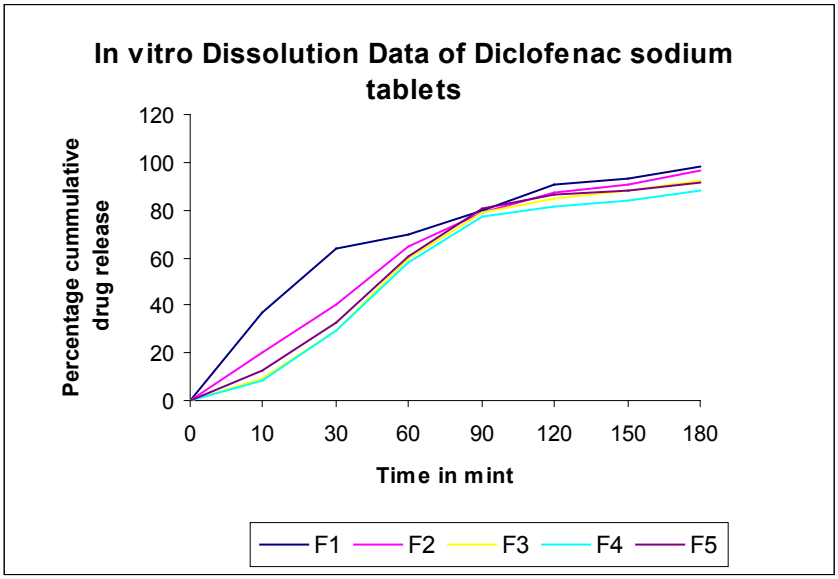


Figure 3: *in vitro dissolution profile of Diclofenac sodium tablets prepared with seed mucilage of Cassia auriculata as binding agent.*

compressibility index values up to 15% result in good to excellent flow properties. All these result indicates that the granules possessed satisfactory flow properties and compressibility. Four batch of tablet were prepared using MCA at four different concentration (0.25, 0.5, 0.75, 1.0%w/v) Guar gum mucilage (1.0 %w/v) was used as standard binder for comparison. The prepared tablets were evaluated for content uniformity, hardness, friability, disintegration time, dissolution profile. All the batches of tablet exhibited good uniformity in content. Hardness of tablet increased with increase in concentration of mucilage. The tablet prepared with 1.0% mucilage of MCA showed the hardness nearly equal to the tablet prepared by using 1.0% w/v of Guar gum mucilage. The percentage friability values were slightly decreased as increase in concentration of mucilage. Through increase in hardness of tablet, increase in concentration interestingly showed decreased in disintegration time of tablet. However this may be due to disintegrant property of such type of mucilage. In vitro dissolution study showed that drug release from the tablets prepared by using mucilage at four different concentrations was more than 85% in 3h (Figure 3).

Table 2: Data Showing, Preliminary Phytochemical Screening of Isolated Mucilage

Active constituent	"NG" Mucilage
Carbohydrate	+
Protein	-
Flavanoids	-
Tannins	-
Saponins	-
Sterols	-
Alkaloids	-
Terpenoids	-

+ Present, - Absent.

Table 3: Physicochemical Properties of MCA

Parameter	Result*
Solubility	Soluble in cold water and warm water; forming viscous colloidal solution, Insoluble in Alcohol, Chloroform, Ethyl acetate & Diethyl ether.
Swelling index (%)\	25.0± 0.15
pH	5.8
Viscosity (0.15%w/v solution)	3.51 cps
Specific gravity (g/ml of 0.15%w/v solution)	0.9975
Loss on drying (%)	8.1 ± 0.02
Total ash (%)	7.72± 0.13
Acid insoluble ash (%)	0.57± 0.05
Water soluble ash (%)	6.532± 0.08

*All values are mean ± S.D. for n=3

Table 5: Technological Characterization of Granules and Tablet, Using MCA as Binder.

Properties	Seed mucilage of *Cassia auriculata* as binder				Guar gum
Concentration	0.25%	0.50%	0.75%	1.00%	1.00%
Percentage of fines (%)	21.50	20.40	19.10	17.40	18.06
Angle of repose	32.56°	30.40°	28.64°	29.42°	25.84°
Mean particle size (mm)	0.34	0.31	0.33	0.32	0.34
Percentage friability (%)	0.75	0.62	0.54	0.46	0.35
Disintegration time in min	8	9	11	14	13
Loose Bulk density (g/cm^2) ± SD	0.576±0.05	0.553±0.03	0.530±0.06	0.513±0.01	0.522±0.04
Tapped bulk density (g/cm^2) ± SD	0.620±0.04	0.607±0.01	0.588±0.02	0.582±0.01	0.580±0.02
Compressibility index (%)	7.09±0.78	7.02±0.24	7.062±0.05	7.01±0.04	7.08±0.07
Content uniformity (%) ± SEM	99.6±0.44	100.2±0.54	100.1±0.52	101.4±0.51	101.0±0.70
Hardness (kg/cm^2) ± SEM	4.90±0.44	5.80±0.04	6.20±0.08	7.10±0.07	6.8±0.10

*All values are mean ± S.D. for n=3

Table 4: Technological Characterization of Microbial load

Natural gum	No. CFU/ ml	Microbial load (No. of CFU / gm of gum)
MCA	12	120

CONCLUSION

The exhausted literature survey conclude before the present work that there is no binding property study on record of this much valued traditional drug. From the above study, it was conclude that Cassia auriculata mucilage can be used as a binder in formulation of uncoated tablets as increase in concentration of mucilage increases the hardness and decrease the disintegration time. This property of mucilage can overcome the friability problems of orodispersible tablets. Moreover it may prove economical as binding property of 1.0% MCA is almost equivalent to 1.0% Guar gum mucilage.

ACKNOWLEDGEMENTS

The author's grateful to the Principal and Management Shree H.N. Shukla Institute of Pharmaceutical Education and Research, Rajkot, Gujarat, for extending laboratory competence and providing necessary amenities to carry out this work.

REFERENCES:

1. Monif T, Mahlhotra AK and Kapoor VP. *Cassiafustula* seed galactomannan: Potential binding agent for pharmaceutical formulation. *Indian J Pharm Sci*. **54**:234–240 (1992).
2. Kapoor VP, Banerji R and Prakash D. Leguminous seeds: Potential industrial sources for gum, fat and protein. *J. Sci Ind Res*. **51**:1–22 (1992).
3. Kakrani HK and Jain NK. A study on binding properties of guggul gum. *Indian J Hosp Pharm*. 100–102 (1981).
4. Bhunvara NS and Khorana ML. Studies on suspending property of mucilages of *hyprophilaspinosa*, *Indian Drugs*. **22**:500–502 (1985).
5. Whistler RL, *Polysaccharides and their Derivatives Industrial gums*, 2nd edition. (Academic Press, New York, 1973) 807.
6. Nandi RC, A Process for preparation of polyose from the seeds of Tamarindus indica, *Ind. Pat*, 142092 (1975).
7. Rao PS and Srivastav HC, *Tamarind Indusrtial gums* In: Whistler R.L., 2nd Edition, (Academic Press, New York, 1973) 369–411.
8. Rao PS. Extraction and purification of tamarind seed polysaccharide. *J Sci Ind Research*. **4**:705 (1946).
9. Willard, Meritt, Dean and Settle, *Instrumental method of analysis*, 7th edition, (CBS Publication New Delhi, 1988) 201–209.
10. Sharma YR, *Element organic Spectroscopy*, 4th edition. (S.Chand Publication, New Delhi, 2009) 132–133.
11. Beckett AH and Stenlake JK, *Practcal Pharmaceutical chemistry, Part 2*. (CBS Publication New Delhi, 2004) 383–389.
12. Tayed PT and Vavia PR. Inclusion complexes of ketoprofen with -cyclodextrin: oral pharmacokinetics of ketoprofen in human *Indian J. Pharm. Sciences*. 408–412 (2005).
13. Murli Mohan GV, Controlled release of diclofenac sodium by gum karaya-chitosan complex coacervate *in vivo* Evaluation. *Indian J. Pharm. Sciences*. 408–412 (2001).
14. Ministry of health and family welfare, *Indian pharmacopeia* 3rd edition. (New Delhi, 1985) A73, A-88.
15. Kokate CK, *Practical Pharmacognosy*, 4th Edition. Vallabh Publication, Delhi; 123 (2006).
16. Michael J and Pelezar JR, *Microbiology*, 5th edition. (Tata, McGraw-Hill Publication, New Delhi, 1993) 126–127.
17. Ministry of health and family welfare, *Indian Pharmacopeia*, (New Delhi, 1996) 2, 710, 711, A-89, A-95, A-114–115, A-80.
18. Gordon RE, Rashanka TW, Fonner DE, Anderson NR and Bankar GS. In: Lachman L., Liberman H.A., and Schwartz J.B. ed. *Pharmaceutical Dosage Forms: Tablets*. Vol. **2**, Marcel Decker, New York; 245 (1999).
19. Banker GS and Neil RA. In; Lachman L., Liberman H.A. and Joseph L.K., ed. *Theory and practices of industrial pharmacy*, 3rd Edition. Varghese publication, mumbai; 297 (1987).
20. Shah D, Shah Y and Rampradhan M. Development and Evaluation of Controlled-Release Diltiazem HCl Microparticles Using Cross-Linked Poly(Vinyl Alcohol). *Drug Develop. Ind. Pharm*. **23**:567 (1997).

21. Aulton ME. Powder flow. In: *Pharmaceutics-The design and manufacture of medicines*. Churchil Livingstone, London New York; 176 (2007).
22. Martin A, Swarbrick J and Cammarata A. Micromeritics. In: *Physical pharmacy and pharmaceutical sciences*, 5th Edition. Wolters Kluwer publication, New Delhi; 492 (2008).
23. Reddy KR, Mutalik S and Reddy S. Once-daily sustained-release matrix tablet of Nicorandil: formulation and *in vitro* Evaluation. *APPS Pharm. Sci. Tech*. **4**(4): (2003).

Figure 1: DSC Thermogram of Diclofenac sodium

Figure 2: DSC Thermogram of NG

Figure 3: in vitro dissolution profile of Diclofenac sodium tablets prepared with seed mucilage of Cassia auriculata as binding agent.

Antioxidant potential of *Trichosanthes dioica* Roxb (fruits)

Yogesh Shivhare[1], Priya Singh[1], Rajak H.[2], Patil U.K.[1], Pawar R.S.[1]*

[1]Department of Pharmacognosy, VNS Institute of Pharmaceutical Education and Research, Berkheda nathu, Neelbud, Bhopal (M.P.) India

[2]Department of Pharmaceutical Sciences, GGDU, Bilaspur, (C.G.) India

Abstract

The study was undertaken to evaluate the antioxidant activity of fruits of *Trichosanthes dioica* (Cucurbitaceae) and compared with ascorbic acid (Standard). Anti-oxidant activity of aqueous extract of *Trichosanthes dioica* (TSD) fruits was studied for its free radical scavenging property in different in vitro methods as 1, 1 diphenyl-2- picryl hydrazyl, nitric oxide, reducing power assay and hydrogen peroxide radical method. Different concentrations of aqueous extract of TSD were prepared and evaluated by standard methods. The IC_{50} values of aqueous extract of TSD were compared with ascorbic acid (Standard) and it was noted that, the extract showed significant concentration dependent free radical scavenging property in all the methods. Results from the study showed that aqueous extract of TSD possess *in vitro* free radical scavenging activity. The findings could justify the inclusion of this plant in the management of antioxidant activity.

Keywords: *Trichosanthes dioica*, antioxidant, DPPH, Reducing power assay.

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** dr_pawar14@rediffmail.com

INTRODUCTION

Free radicals can be described as chemical species that have an unpaired electron and play very important role in human health and beneficial in combating against several diseases. These free radicals are highly unstable and when the amount of these free radicals exceed in the body, it can damage the cells and tissues and may involved in several diseases like cardiovascular disorders, lung damage, inflammation etc. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals (1–2).

Trichosanthes dioica (family- Cucurbitaceae) is a well known plant commonly called as Parwal in Hindi, Potol in Bengal, and Palwal in Punjab. The medicinal attributes *T. dioica* have been known since time immemorial. The plant is alternative, tonic, useful in obstinate fevers, boils etc. The roots are cathartics. The leaves are anthelmentic. The fruits are sweet, cardiotonic, appetizer and stomachic. The plant *T. dioica* possesses many medicinal properties including laxative, cooling, febrifuge, cathartic etc. (3–5). However relevant experimental work has not yet been explored. Therefore, the aim of this study was to evaluate the antioxidant activity of aqueous extract of *Trichosanthes dioica* Roxb fruits.

MATERIALS AND METHODS

Plant material and extraction

Fresh unripe fruits of *Trichosanthes dioica* (2 kg) were purchased from the local market of Bhopal, India, in the month of August 2008. The plant was authenticated by Dr. A.S.Yadav, Professor, Government MVM College, Bhopal. The collected fruits were cut into small pieces and were shade dried. The dried pieces were pulverized into moderately coarse powder and stored in well closed container. The shade dried powder of TSD fruits (125g) were macerated with water for 72 hrs then concentrated and dried under reduced pressure to semisolid mass and residue was obtained (16.96g yield w/w). The residue was stored in a desiccator.

Qualitative test analysis

Qualitative test analysis was performed to determine chemical constituents present in the dried aqueous extract of TSD. The extract was tested for the presence of various

phytoconstituents viz. alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds and tannins (6–7).

DPPH scavenging activity

The procedure of Brand-Williams(8) has been adapted for evaluation of the free radical scavenging capacity of the aqueous extract. Different concentrations (05–45µg/ml) of aqueous extract of TSD were prepared in suitable solvent and 3ml of each solution was mixed with 1 ml of a 0.1mM DPPH solution. The decrease in absorbance was measured at 515 nm after 30 minutes of incubation period at room temperature using a UV Visible spectrophotometer 1700 (Shimadzu). The scavenging activity of sample extract was expressed as the inhibition of DPPH radical and calculated according to the following formula with as the control:

Scavenging Activity (%) = [(A control – A sample) / A control]*100, where $A_{control}$ (containing DPPH solution) and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Reducing power assay

Reducing power of aqueous extract of TSD was estimated using the protocol reported by Oyaizu (9). Different concentrations of aqueous extract of TSD (5–25µg/ml) were prepared and 1ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. To this mixture, 2.5 ml of 10% trichloroacetic acid (TCA) was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and $Fecl_3$ (0.5 ml, 0.1%) was added and the absorbance was measured at 700 nm.

The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was evaluated by the method of Gupta (10). 1ml of Sodium nitroprusside (10mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different concentrations (20 – 120µg/ml) of the aqueous extract of TSD dissolved in water and incubated at 25^0 C for 180 min. The samples from the above were reacted with equal volume of Greiss reagent (1% sulphanilamide, 0.1% napthylethylenediamine hydrochloride and 3% of phosphoric acid). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Hydrogen peroxide radical scavenging activity

The ability of the aqueous extract of TSD to scavenge hydrogen peroxide was determined according to the method of Ruch (11). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a UV Visible spectrophotometer 1700. Then hydrogen peroxide solution (0.6 ml, 40 mM) was mixed to different concentrations (30 – 180µg/ml) of the extract dissolved in water. The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

The percentage scavenging was calculated by using the formula

$(A_{control} - A_{sample})/ A_{control} \times 100$, where $A_{control}$ is the absorbance of solution without extract and A_{sample} is the Absorbance with different dilutions of drug extract. Ascorbic acid was used as reference standard.

Statistical Analysis

Data are presented as the mean ± SEM of each triplicate test. The analysis was performed by using Dunnett vs. Control test and by ANOVA. P‘0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemicals

The phytochemical screening of aqueous extract of TDS revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds and tannins.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of the aqueous extract of TSD with IC_{50} (% Inhibition) are shown in fig 1.1. The IC_{50} value of aqueous extract of TSD and

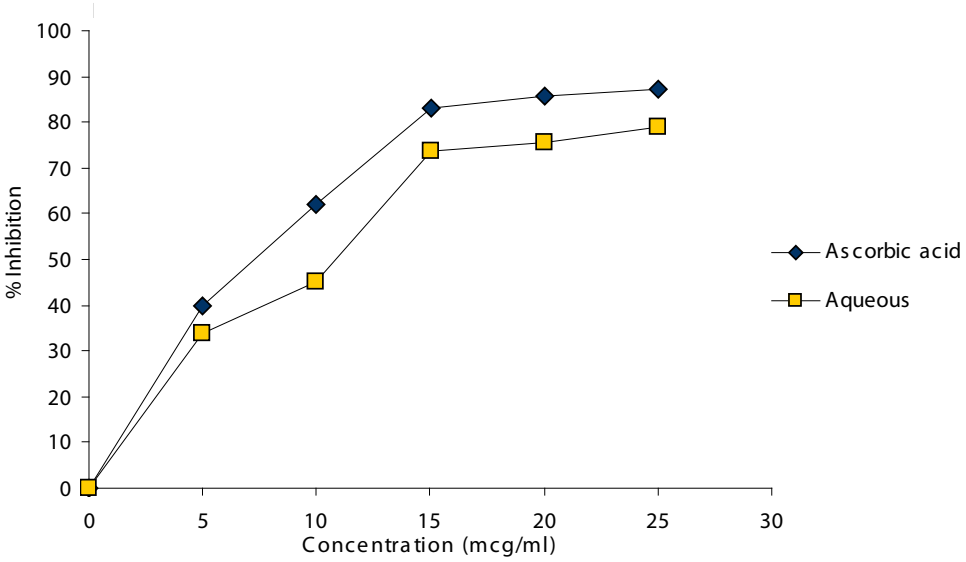


Figure 1.1 Effect of aqueous extract of *Tricho santhes* dioia on DPPH scavenging activity

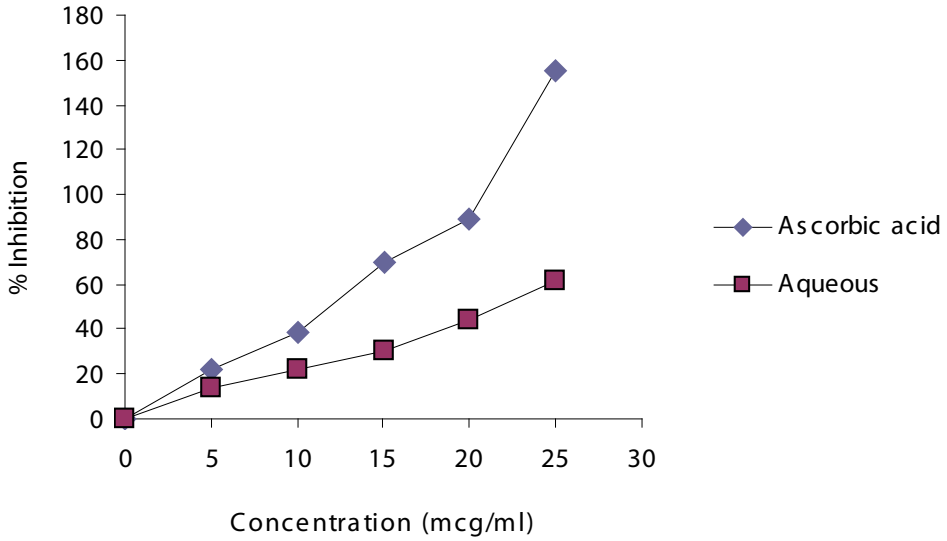


Figure 1.2 Effect of aqueous extract of *Trichosanthes dioica* on reducing power assay

standard (ascorbic acid) were found to be 33µg/ml, and 11µg/ml, respectively. The results showed a significant ($p<0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of aqueous extract as compared to standard (ascorbic acid).

Reducing power assay

The Reducing power of aqueous extract of TSD and ascorbic acid were shown in the fig 1.2 The IC_{50} value of aqueous extract of TSD and ascorbic acid (standard) were found to be 25µg/ml and 12µg/ml, respectively.

Nitric oxide scavenging activity

The nitric oxide scavenging activity of aqueous extract of TSD and ascorbic acid were shown in the fig 1.3 which illustrates the % inhibition of nitric oxide generation by aqueous extract of TSD. The IC_{50} value of aqueous extract and ascorbic acid (standard) were found to be 112µg/ml

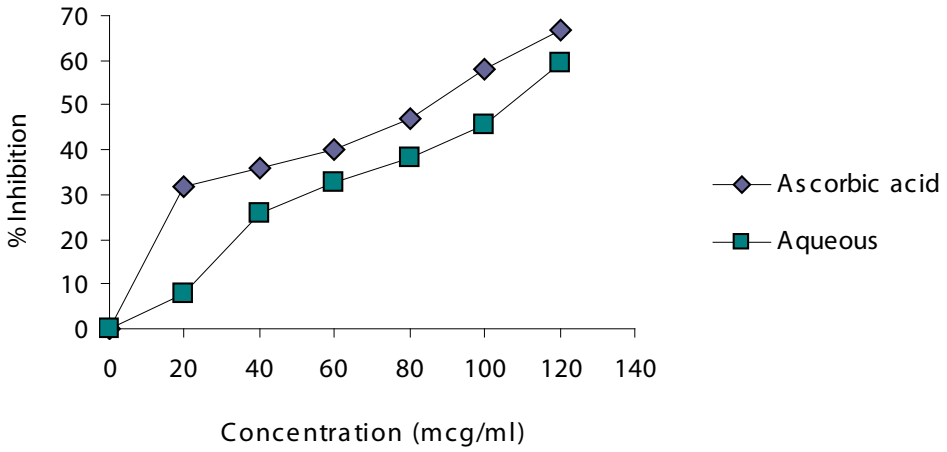


Figure 1.3 Effect of aqueous extract of *Tricho diocia* on No scavenging activity

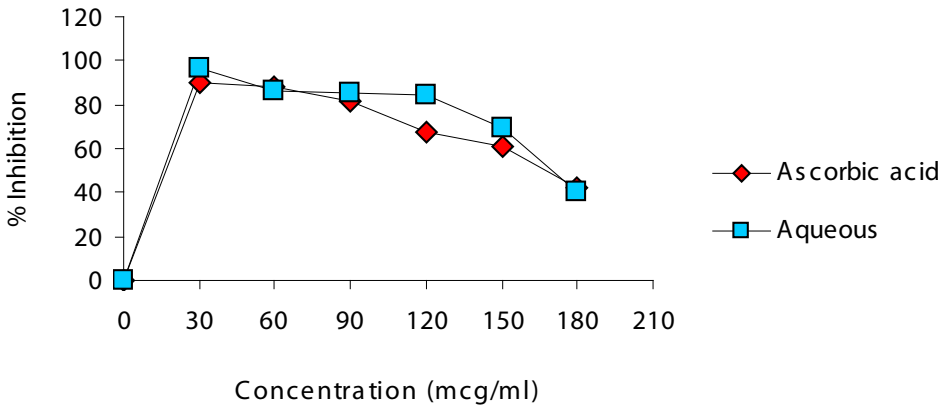


Figure 1.4 Effect of aqueous extract of *Tricho dioica* on H_2O_2 radical scavengin activity

and 85µg/ml, respectively. The results indicate significant ($p<0.01$) decrease in the concentration of nitric oxide radical due to the scavenging ability of aqueous extract as compared to standard.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide scavenging activity of aqueous extract of TSD and ascorbic acid were shown in the fig 1.4. It showed significant scavenging activity of hydroxyl radical generated from H_2O_2 system. The IC_{50} value for aqueous extract was 171µg/ml, whereas 167µg/ml was the value of ascorbic acid. The results indicated that aqueous extract of TSD possessed significant antioxidant activity ($p<0.01$).

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

Aqueous extract of TSD showed the presence of flavonoids and phenolic compounds. Since the antioxidant activities of these constituents from plant origin have already been established (12),[22] it can speculate that these constituents may be responsible for the observed antioxidant effects.

DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the *in vitro* antioxidant activity of crude plant extracts (13–14). A DPPH radical scavenging ability of the extract was significantly lower than those of ascorbic acid. It was evident that the extract did show the proton donating ability and could serve as free radical

inhibitor or scavenger, acting possibly as primary antioxidants.

In the present study, the reductive capacity of the aqueous extract of TSD was compared with ascorbic acid (Standard). Significant antioxidant potential of any compound depends on its reducing capacity (15). The reducing capacity of the aqueous extract of TSD was found to be concentration dependent and showed significant potential.

Nitric oxide is a short-lived (half-life 3–30 s) colorless gas that is moderately soluble in water highly soluble in organic solvents (16). It is an important chemical mediator or essential bioregulatory molecule which is generated by neurons, endothelial cells etc and required for several physiological processes like immune response, neural signal transmission and control of blood pressure. Several diseases occur due to excess concentration of nitric oxide (17–18). Oxygen reacts with the excess nitric oxide to generate nitrites and anions which act as free radicals (19–20). In this study, the fruits of plant TSD compete with oxygen to react with nitric oxide and thus inhibit generation of anions.

Hydroxyl radicals are the major active species causing lipid oxidation and enormous biological and cellular damages (21). Its ability to produce active oxygen species is due to its ability to generate highly reactive hydroxyl radical through the Fenton reaction (22). As the aqueous extract scavenged hydrogen peroxide radical similar to the standard (ascorbic acid) so it reflects that the plant extract could possibly inhibit the formation of hydroxyl radical.

CONCLUSION

In this study, the present results indicates that the aqueous extract of TSD possess antioxidant properties due to the presence of phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study has to some extent validated the medicinal value of the fruits of *Trichosanthes dioica*.

REFERENCES:

1. Upadhye M., Dhiman A. and Shriwaikar A.. Antioxidant activity of aqueous extract of *Holostemma Annulare* (Roxb) K. Schum. *Adv. Pharmacol. Toxicol.*; **10** (1): 127–131 (2009).
2. Mishra J., Srivastava R.K., Shukla S.V., Raghav C.S.. Antioxidants in Aromatic and Medicinal plants. *Science tech. entrepreneur*. 1–16 (2007).
3. Kirtikar K.R. and Basu B.D.. *Indian Medicinal Plants. II*: 2nd ed M/s Singh Bishwas. Nirali prakashan Dehradun; 1110 (1976).
4. Chaterjee A. and Pakrashi S.C.. The treatise of Indian Medicinal Plants; **5**: 137 (2003).
5. Chopra R.N., Nayar S.L. and Chopra I.C.. *Glossary of Indian Medicinal Plants*, 248.
6. Khandelwal K.R.. *Practical Pharmacognosy*. 9 edition. Nirali Prakashan; 149–153 (2002).
7. Ansari S.H.. *Essentials of Pharmacognosy*. Birla Publication Pvt. Ltd. New Delhi 1 edition; 367–369 (2005–2006).
8. Brand William W., Cuvelier M.E., Berset C.. Use of free radical method to evaluate antioxidant activity. *Food Science Techno.*; **28**(1):25–30 (1995).
9. Oyaizu M.. Studies on product of browning reaction prepared from glucose amine. *Japanese J Nut*; **44**: 307–315 (1986).
10. Gupta M., Kanti M.U., Gomathi P.. In vitro antioxidant and free radical scavenging activities of *Galega Purpurea* root. *Phcog. Mag.*; **3**(12): 218–224 (2007).
11. Ruch R.J., Cheng SJ, Klauning JE. Prevention of cytoxicity and inhibition of intercellular communication antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*; **10**: 1003–1008 (1989).
12. Di Carlo G., Mascolo N., Izzo A.A., Capasso F.. Flavonoids: Old and new aspects of a class of natural therapeutic drugs. *Life Sci.*; **65**:337–53(1999).
13. Navarro M.C., Montilla MP, Martin A, Jimenez J, Utrilla MP. Free radicals and antihepatotoxic activity of *Rosmarinus tomentosus*. *Planta Med.*; **59**: 312–14 (1992).
14. Thabrew M.I., Hughes R.D., MC. Farlane IG.. Antioxidant activity of *Osbeckia aspera*. *Phytother Res.*; **12**: 288–90(1998).
15. Zhang Wei-Min, Han Bin li lin, De Zhang Hai. Antioxidant activities of extracts from *Areca (Areca catetu L.)* Flower, Husk,and Seed. *Electronic Journal of environmental, agricultural and food chem.*; **8** (9) 740–748 (2009).
16. Ignarro L.J., Byrns R.E., Buga G.M., Wood K.S.. Endothelium derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. *Circ Res.*; **61**: 866–879 (1987).
17. Moncada Ialenti S., Di. Rosa M.. Modulation of adjuvant arthritis by endogenous nitric oxide. *British J. Pharmacol.*; **110**: 701–705 (1993).
18. Ross R.. The pathogenesis of atherosclerosis: a perspective for the 1990's. *Nature*; **362**: 801 (1993).
19. Cotran R.S., Kumar V., Robbin's Collins T.. *Pathological basis of diseases*; 6th ed Thomson Press, Noida: 1–43 (1999).
20. Aruoma O.I., Kaur H., Halliwell B.. Oxygen free radicals and human diseases. *J. The Royal Soc. Health*; **111**: 172–77 (1991).
21. Milic B., Djilas S.M., Canadanovic-Brunet J.M.. Antioxidative activity of phenolic compounds on the metal into break down of lipid peroxidation system. *Food Chem.*; **61**: 443–47 (1998).
22. Namiki M.. Antioxidants / Antimutagens in food. *Critical Review of Food Science and Nutrition*; **29**: 273–300 (1990).

Comparative Phytochemical and Antibacterial Studies on the bark of *Alstonia scholaris* R.Br. and *Alstonia macrophylla* Wall. ex G.Don

M. S. Khyade[1] and N. P. Vaikos[2]

[1]Post Graduate Department of Botany, Sangamner Nagarpalika Arts, D.J. Malpani Commerce and B.N. Sarda Science College, Sangamner, Dist. Ahmednagar-422 605 (MS), India.
[2]Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (MS), India

ABSTRACT

Comparative phytochemical and antibacterial activities of bark of *Alstonia scholaris* and *Alstonia macrophylla* were investigated. The successive different solvent extracts showed the presence of alkaloids, phenolics, saponins and tannins were found in both the species. The antibacterial activities of bark of *A. scholaris* and *A. macrophylla* in successive different solvent were tested against gram +ve and gram –ve organisms. The chloroform extracts of *A. macrophylla* showed broader spectrum of antibacterial activity when compared with *A. scholaris*. However. *Alstonia scholaris* is widely used medicinal plant.

Keywords: Comparative, bark, *Alstonia* species, phytochemical, antibacterial
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** mskhyade@rediffmail.com

INTRODUCTION

Green plants represent a reservoir of effective chemotherapeutics and can provide valuable source of natural antimicrobials (1, 2). Plant species which have one or more of its organs containing substances that can be used for therapeutic purpose are called medicinal plants (3).Plants have been used as medicinal agents from the earliest day of mans existence (4, 5) and has made it necessary to study them in details in order to discriminate the kinds employed for different purposes (6). In particular, the antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food formation, pharmaceuticals, alternative medicine and natural therapies (7).

Now a day, infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and Governments all over the world, especially with the current increasing trends of multidrug resistance among emerging –reemerging bacterial pathogens to the available modern drug or antibiotics (8, 9). It is therefore very necessary that the search for newer antibiotic serves to be a continued process. Plants are the cheapest and safer alternative source of antimicrobials (10, 11). Therefore, with increasing acceptance of traditional medicine as an alternative form of health care, the screening of plants for active compounds has become very important.

Alstonia scholaris R. Br. (Apocynaceae), popularly known as "Saptaparni" or "Devil's tree", and *A. macrophylla,* are used in the traditional systems of medicine. However, the former one is widely recognized medicinal plant.

The bark of *A. scholaris* is bitter, tonic, astringent, expectorant, alterative, anthelmintic, emmenagogue and galactogogue. It has proved valuable in fever, chronic diarrhea and in advanced stages of dysentery (12-15). Whereas the bark of *Alstonia macrophylla* is used for the same purpose as that of *Alstonia scholaris* (16). Decoction of the stem bark is effective in stomach ache, skin diseases and urinary infections (17). Moreover, phytochemistry on these two species studied earlier (18-21). The genus *Alstonia* has been the subject of antimicrobial activities (22, 23). This work, however, is designed to evaluate the comparative account of antibacterial activity of related species.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

The fresh barks of *A. scholaris* R. Br. were collected in the month of October 2005 from forest area of Aurangabad

district (M.S.) and that of *Alsotia macrophylla* from the Botanical Garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The plant species were identified with the help of Flora of Marathwada (24) and voucher specimens have been deposited at the Botany department of the university. Plant samples were washed, shade dried at room temperature for 15 days.

PREPARATION OF EXTRACTS AND PHYTOCHEMICAL SCREENING

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 gm of powdered material was extracted in soxhlet extraction apparatus successively with 250 ml of each of the following solvents. Petroleum ether, chloroform, acetone and methanol (25). The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40°C) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator and were suspended in dimethyl sulphoxide (DMSO) for prior to use (26).

Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (27-30). The positive tests were noted as weak (+), moderate (++), strong (+++) and absent (-).

TEST CULTURE

The test bacteria used for the screening antimicrobial activity were *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* MTCC 106 *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* MTCC 2488, *Klebsiella planticola* and *Bacillus megaterium*. The cultures were obtained from Microbial Type culture Collection (MTCC), IMTEC, Chandigarh, India. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were checked for viability and purity by regular plating. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37±1°C for 24 hours.

SCREENING FOR ANTIBACTERIAL PROPERTIES

The antibacterial activities of the successive bark extracts of petroleum ether, chloroform, acetone and methanol of both the plant species were tested by Agar well diffusion method (31). The culture plates were prepared by pouring 20 ml of sterile nutrient agar. 1ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (6 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 100mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37°C during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Ampicillin (40 µg/ml) was used as standard antibiotics. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the bark of both species of *Alstonia* are shown in Table 1. It indicates the presence of alkaloids, phenolics, saponins and tannins. The presence of steroids and terpenoids are indicated only in *Alstonia scholaris* while cardiac glycosides and flavonoids are found alone in *A. macrophylla*. However there were complete absence of anthraquinones and leucoanthocyanins in both species.

The antibacterial activities of *A. scholaris* and *A. macrophylla in* petroleum ether, chloroform, acetone and methanol extracts against bacteria examined in the current study and their potency were qualitatively assessed by the presence or absence of inhibition zones and zone diameter (Table 2). The results showed that the extracts of *A. scholaris* mediated some degree of activity against bacteria. Except, *S. aureus* and *S. typhi* other strains like *M. luteus, B.subtilis, B. megaterium, K. planticola E. coli* and *P. aeruginosa* are inhibited by acetone extracts only, while other extracts such as petroleum ether, chloroform and methanol showed negative inhibition at test concentration.

The antibacterial activity of *A. macrophylla* extracts were the most prominent in activity against all bacteria tested at test concentration (table 2). Except petroleum ether extracts, others such as chloroform, acetone and methanol showed broader spectrum of activity. However, chloroform extracts of *A. macrophylla* exhibited significant antibacterial activities against all the bacteria tested but, particularly, to *B. subtilis, S.aureus* and *E. coli* with a diameter same or greater than standard antibiotic ampicillin.

Based on these results, it is possible to conclude that of two species of *Alstonia*. *A. macrophylla* exhibited broader range of antibacterial activity to varying degrees, as it is a less known medicinal plant in the Indian literature. Particularly, the chloroform extracts of *A. macrophylla*

Table-1: Phytochemical constituents of bark extracts of *A.scholaris* and *A. macrophylla*

	Alstonia scholaris				*Alstonia macrophylla*			
Phytochemicals	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Alkaloids	—	+++	—	+++	—	+++	—	+++
Anthraquinones	—	—	—	—	—	—	—	—
Cardiac glycosides	—	—	—	—	—	—	++	+++
Coumarins	—	—	—	—	—	—	—	—
Flavonoids	—	—	—	—	—	++	++	+++
Leucoanthocyanins	—	—	—	—	—	—	—	—
Simple phenolics	—	—	+	++	—	++	++	+++
Steroids	—	—	—	++	—	—	—	—
Saponins	—	—	+	++	—	++	++	+++
Tannins	—	—	+	++	—	+	++	+++
Terpenoids	—	++	++	++	—	—	—	—

Note: - A - Pet ether, B - Chloroform, C - Acetone, D - Methanol, (+) - weak, (++) - moderate, (+++) - strong and (-) - absent.

Table-2: Antibacterial activity of bark extracts of *A. scholaris* and *A. macrophylla*

Organisms	**Gram stain + / –**	**Inhibition zone in diameters (mm / sensitive strains)**									
		A. scholaris				***A. macrophylla***				**DMSO**	**Ampicillin**
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**		
Staphylococcus aureus	+	—	—	—	—	—	24	16	15	—	23
Bacillus subtilis	+	—	—	11	—	—	32	16	18	—	21
Bacillus megaterium	+	—	—	11	—	—	10	15	15	—	25
Micrococcus luteus	+	—	—	13	—	—	27	15	17	—	30
Escherichia coli	–	—	—	10	—	—	18	15	17	—	17
Salmonella typhi	–	—	—	—	—	—	11	13	10	—	19
Pseudomonas aeruginosa	–	—	—	10	—	—	—	14	14	—	16
Klebsiella planticola	–	—	—	11	—	—	22	18	15	—	21

Note: - A - Petroleum ether, B - Chloroform, C - Acetone, D - Methanol, — no inhibition, Figures are diameter of zone of inhibition.

showed significant antibacterial activities and could be used as antimicrobial agents in new drugs for therapy.

ACKNOWLEDGEMENT

The authors wish to thank Head of Botany Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing the necessary laboratory facilities.

REFERENCES

1. Balandrin MF, Klocke JA, Wutule ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. *Science* **228**: 1154–1160 (1985).
2. Satish H, Raveesha KA, Janardhana GR. Antibacterial activity of selected Peruvian medicinal plants. *J Ethnopharmacol* **88**: 199–204 (1999).
3. Sofowora A. *Medicinal plants and Traditional Medicine in Africa* (John Willy. New York, 1982) 289.
4. Hansch C. *Comprehensive Medicinal Chemistry* Vol. **1**. (Pergamon Press, (1990)
5. Shellard EJ. Medicines from plants. *Plant Med* **53**: 6–8 (1987).
6. Ghani A. *Medicinal Plants and Traditional Medicinal Portions: Problems and Prospects of their Standardization*. (The state of medicinal plant research in Nigeria, 1986) 65.
7. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *App. Microbiol* **86**: 985–990 (1999)
8. Franklin TJ, Snow CA. *Biochemistry of antimicrobial action* 4th edn. (Chapman and Hall New York, 1989) 134–155.
9. Prescott L, Harley J, Klein DA. *Microbiology* 5th edn. (Mc-Graw Hill London, 2002) 820–950.
10. Pretorius CJ, Watt E. Purification and identification of active components of *Carpobrotus edulis* L. *J Ethnopharmacol* **76**: 87–91 (2001).

11. Sharif MDM, Banik GR. Status and Utilization of Medicinal Plants in Rangamati of Bangladesh. *Res J Agri Biol Sci* **2**: 268–273 (2006).
12. Chopra RN, Nayar SI, Chopra IC. *Glossary of Indian Medicinal Plants*. (CSIR, New Delhi, India, 1956) 13–14.
13. Dastur JF. *Medicinal plants of India and Pakistan* (Taraporewala and Co. Pvt. Ltd., Bombay, 1962) 18.
14. Joshi SG. *Medicinal plants* (Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, 2000) 40–41.
15. Siwakoti M, Siwakoti S. Ethnobotanical uses of plants among the star tribe of Nepal. In *Ethnobotany and medicinal plants of Indian subcontinent*. (Maheshwari JK Editor, Scientific Publishers, Jodhpur, India, 2000) 99–108.
16. Anonymous. *The useful plants of India* (Publications and information Directorate, Council of Scientific and Industrial Research, New Delhi, 1992)
17. Bhargava N. Ethanobotanical studies of the tribes of Andaman and Nicobar Islands, India. I onge. *Eco. Bot* **37**: 110–119 (1983).
18. Chakravarti D, Chakravarti RN, Ghose R. Triterpenes of *Alstonia scholaris* (dita-bark). *Experientia* **13**: 277 (1957)
19. Hadi Surya, Bremner John B. Initial studies on Alkaloids from Lombok Medicinal Plants. *Molecules* **6**: 117–129 (2001).
20. Kam TS, Nyeoh KT, Sim KM, Yoganathan K. Alkaloids from *Alstonia scholaris*. *Phytochemistry* **45**: 1303–1305 (1997).
21. Salim AA, Garson MJ, Craik DJ. New indole alkaloids from the bark of *Alstonia scholaris*. *J Nat Prod* **67**: 1591–1594 (2004).
22. Chattopadhyay D, Maiti K, Kundu AP, Chakraborty MS, Bharda R, Mandal SC, Mandal AB. Antimicrobial activity of *Alstonia macrophylla*: a folklore of bay islands. *J. Ethnopharmacology* **77**: 49–55 (2001).
23. Khan M, Omaloso R, Kihara M. Antibacterial activity of *Alstonia scholaris* and *Leea tetramera*. *Fitoterapia* **74**: 736–40 (2003).
24. Naik VN. *Flora of Marathwada* (Amrut Prakashan, Aurangabad (M.S), India, 1998) 528–529.
25. Vogel AI. *Elementary practical organic chemistry* (2 edition), (Orient Longman Limited, 1988) 45–168.
26. Beyer H, Walter W. *Organic chemistry*, 4th ed. (Published by Jerry March, 1997) 146–148.
27. Harborne JB. *Phytochemical methods* (Chapman Hall London, 1984) 100–101.
28. Trease E, Evans WC. 1987. *Pharmacognosy*, Billiare Tindall, London.
29. Ajaiyeoba EO. Phytochemical and antimicrobial studies of *Gynandropsis gynandra* and *Buchholzia coriaceae* extracts. *African Journal of Biomedical Research* **3**: 161–165 (2000).
30. Edeoga, HO, Okwu DE, Mbacbie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J Biotechnol*. **4**: 685–688 (2005)
31. Kavanagh F. *Analytical microbiology* Part II. (New York Academic Press, 1972) 126.

Pharmacognostic And Physio-Chemical Studies On The Leaves Of *Cardiospermum halicacabum* L.

Patil, A. G.*, Joshi, K. A., Patil, D. A., Phatak, A. V., Naresh, Chandra

Department of Botany-Herbal Sciences, Birla College, Kalyan-421304, M.S., India

ABSTRACT

Cardiospermum halicacabum L. (Sapindaceae) commonly known as "Balloon vine" is a dioecious, hairy, climbing vine with balloon like clusters of white flowers framed by finely dissected, delicate foliage. The leaf has a bitter taste; the entire plant is used as anti-inflammatory, antibiotic against many bacteria, antiparasitic, antipyretic and as an analgesic. The ethanolic extract of leaves exhibits significant anti-arthritic effect. The present study was therefore carried out to provide requisite pharmacognostic details about the leaf. Pharmacognostic investigation of the leaf and leaf powder of *Cardiospermum halicacabum* L. was carried out to determine its morphological, anatomical and phytochemical diagnostic features. The preliminary phytochemical analysis and Thin Layer Chromatography has been performed. The leaf was also characterized for its physico-chemical properties. The presence of covering trichomes and anomocytic type of stomata are the characteristic features observed in the microscopy of leaf. Preliminary phytochemical analysis indicated presence of tannins, saponins, flavonoids, glycosides and cardiac glycosides. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: *Cardiospermum halicacabum* L., Pharmacognosy, Physicochemical analysis.

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** dravinashpatil@rediffmail.com

INTRODUCTION:

Traditional knowledge of plants is responsible for most of the medicine and food used in modern society. The exploration of traditional knowledge for cures to common diseases is attractive, but also overwhelming. *Cardiospermum halicacabum* L., commonly known as Balloon vine, is an important medicinal herb belonging to family Sapindaceae. The plant is a dioecious, hairy, climbing vine with clusters of white flowers, finely dissected, delicate foliage and balloon like fruits. The root of the plant is considered as diaphoretic, diuretic and aperient. It is also administered in fever. The whole plant is applied to reduce swellings and hardened tumors (1). There is a claim that it is used by some locals to treat rheumatoid arthritis in Asian and African communities. This claim is substantiated by the researchers (2, 3).

The whole plant has been used as anti-inflammatory (4, 5), as an antibiotic against many bacteria such as *Escherichia coli, Salmonella typhi* etc. (4, 6), as an antipyretic (4, 7), antiparasitic (8), as an effective non toxic antifertility herb (9) and as analgesic (4). Eswar Kumar *et al*.(10) reported that the ethanolic extract of leaves exhibits significant anti-arthritic effect.

In the global market, balloon vine has been utilized in several products, 'Love in a puff', 'Balloon Vine' and 'Heartseed'. It is also one of the ingredients in "Allergy Relief Liquid ™" and "Bioforce Pollinoson® Tabs" marketed by Bioforce USA as a natural relief for hay fever, allergies, sneezing, watery eyes, and allergic reactions. Another US based company, Boericke and Tafel produces "Florasone Cardiospermum Cream" for skin ailments such as swelling, scaling, blisters/vesicles, burning and pain. These products are supported by the various claims concerning the many medicinal properties of balloon vine (11).

Therefore the present investigation of *Cardiospermum halicacabum* L. leaves is taken up to establish pharmacognostic profile of the leaves which will help in crude drug identification as well as in standardization of the quality and purity.

MATERIALS AND METHODS

Herbarium of *Cardiospermum halicacabum* was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. Fresh leaves of *Cardiospermum halicacabum* were collected from Haji Malang (Kalyan, M.S., India), washed under running tap water and blotted dry for further studies. The leaves were dried in preset

oven at 40 ± 2°C for about two weeks, ground into powder and used for further analysis. Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described by Mukherjee (12). Preliminary phytochemical analysis of powdered leaf was performed as described by Khandelwal (13) and Kokate (14). Phytochemical analysis was carried out using Thin Layer Chromatography as described by Wagner and Bladt (15). Fluorescence analysis was conducted using methods of Kokoski (16) and Chase and Pratt (17).

RESULTS

Macroscopic Characters (Plate 1 A, 1 B)

Color: Green.

Size and Shape: Petiole: 1.7-3.4 cm long, ultimate segments of the leaves 2.7 – 4.0 cm in length, lanceolate.

Texture: Sparsely pubescent.

Extra features: Leaves alternate and compound, deltoid, 2-ternate, serrate, very acute at the apex and narrowed at the base.

Microscopy

Surface preparation of leaves revealed presence of covering unicellular trichomes and anomocytic type of stomata (Plate 1 G, 1 H).

Transverse section of leaf:

It is dorsiventral leaf. Following tissues are present in midrib and lamina.

Midrib:

The midrib is broadly hemispherical on the abaxial side with short lump on the adaxial side. Covering unicellular trichomes are present on either sides of midrib. Inverted vascular bundles (i.e., xylem towards the dorsal surface and phloem towards the ventral surface) is the characteristic of the leaf (Plate 1 C).

Lamina:

The lamina of the leaf shows upper epidermis, mesophyll and lower epidermis. Upper epidermis is composed of single layer of rectangular cells covered with cuticle. It also shows presence of covering unicellular trichomes. Mesophyll is differentiated into 1-2 layers of palisade followed by 3-4 layered spongy parenchyma. Lower epidermis consists of single layer of rectangular cells (Plate 1 D).

Transverse section of petiole

Transverse section of petiole shows two prominent grooves towards upper side whereas the lower side is round. The epidermis is composed of single layer of cells. Few trichomes are observed on epidermal cells, which are identical with that of leaf. Chlorenchymatous hypodermis is present below grooves. 4-6 vascular bundles are present in the ground tissue. Each vascular bundle is collateral. The xylem is found towards upper side and the phloem lies towards lower side. The remaining portion of the ground tissue is composed of parenchyma (Plate 1 E).

Transverse section of rachis

Transverse section of rachis is polygonal in outline with two winged projections at the upper side. Epidermis is single layered and is composed of horizontally flattened cells. The cells are compactly arranged and showed presence of cuticle. Covering unicellular trichomes are present on epidermal cells. The peripheral layers in the ground tissue are composed of collenchymatous and chlorenchymatous cells. These cells occur as bands and they alternate with each other thus forming a continuous layer next to epidermis. This forms the hypodermis. A continuous ring of pericyclic fibers is present in the ground tissue. Vascular bundles are arranged in a ring in the ground tissue and are collateral. The rest of ground tissue is parenchymatous (Plate 1 F).

Powder characteristics: Preliminary examination of powder

Leaf powder is green in color with characteristic odor and smooth texture.

Microscopic examination of powder

The various diagnostic characteristics of powdered leaf are shown in Table -1.

Quantitative determination

The number of stomata, vein islet number, measurement of stomatal index and size of stomata were done with the

Table - 1: Microscopical features of the powdered leaves of *Cardiospermum halicacabum* L.

Observations	Plate 1
Covering unicellular trichomes.	I
Spiral thickening	J
Leaf surface showing stomata	K
Fibers	L

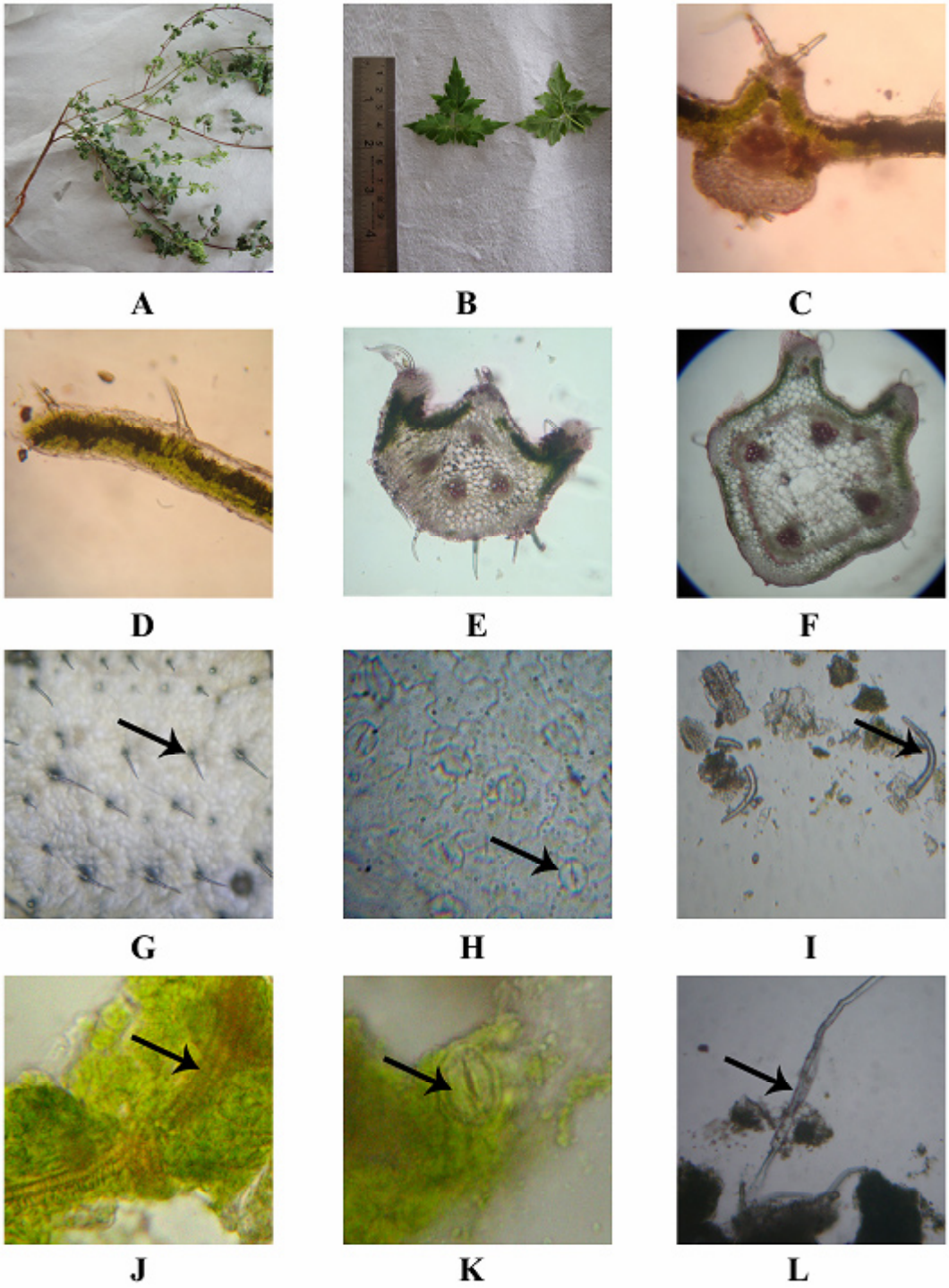


Plate No.1: Macroscopic, microscopic and powder characteristics of *Cardiospermum halicacabum* Linn.

Table 2: Quantitative leaf microscopy of *Cardiospermum halicacabum* L.

Parameter	Value
Stomatal index	33.6
Number of Stomata	20
Vein islet number	17
Stomatal size	
Length (µm)	15.6 – 20.8
Breadth (µm)	7.8 – 13

Table 3: Physico–;chemical studies of *Cardiospermum halicacabum* L. leaves

Parameter	Observation
Ash values	
a. Total ash content (%)	12.9
b. Acid insoluble ash (%)	1.5
c. Water soluble ash (%)	4.3
Extractive values	
a. Water soluble extractive values (%)	18.08
b. Alcohol soluble extractive values (%)	19.84
Loss on drying (%)	56.18

help of calibrated ocular micrometer. Values are tabulated in Table - 2.

Physico-chemical Parameters

The data on ash values are indicative of the purity of drug, extractive values are representative of the presence of polar or non-polar compounds and loss on drying value also indicates that where the drug is safe regarding any growth of bacteria, fungi and yeast (18). Loss on drying, percentage of total ash, acid insoluble ash, water soluble ash and different extractive values are tabulated in Table - 3.

Table - 4: Preliminary phytochemical screening of *Cardiospermum halicacabum* L. leaves

Tests for Phytoconstituents	WE	AE	CE
Carbohydrate	+	+	+
Proteins	+	–;	–;
Amino acid	+	–;	–;
Saponins	+	–;	–;
Tannins	+	+	+
Hydrolysable Tannins	–;	–;	+
Flavanoid	+	–;	–;
Steroid	–;	+	+
Glycosides	+	–;	–;
Cardiac glycosides	+	+	+
Anthraquinone	–;	–;	–;
Volatile oil	–;	–;	–;

WE: Water Extract
AE: Alcohol Extract
CE: Chloroform Extract
+ : Present
–; : Absent

Phytochemical Evaluation

Preliminary phytochemical screening is tabulated in Table - 4.

Table 5: Chromatographic result of *Cardiospermum halicacabum* L. leaf extract:

Compound	Extract	Number of Spots	Rf value
Arbutin	Methanolic	4	0.05, 0.13, 0.44, 0.50
	Aqueous	2	0.05, 0.11
Cardiac glycoside	Methanolic	5	0.11, 0.26, 0.62, 0.75, 0.91
	Aqueous	1	0.15
Essential oil	Methanolic	9	0.15, 0.26, 0.29, 0.39, 0.42, 0.53, 0.57, 0.89, 0.94
	Aqueous	-	-
Bitter principle	Methanolic	5	0.16, 0.30, 0.42, 0.68, 0.77
	Aqueous	2	0.21, 0.47
Pungent principle	Methanolic	5	0.52, 0.63, 0.76, 0.84, 0.90
	Aqueous	-	-
Anthracene	Methanolic	4	0.25, 0.53, 0.58, 0.93
	Aqueous	1	0.28
Saponin	Methanolic	3	0.29, 0.48, 0.85
	Aqueous	1	0.57

Table 6: Fluorescence analysis of *Cardiospermum halicacabum* L. leaves

Treatment	Observation under		
		UV light	
	Ordinary light	254 nm	366 nm
Powder as such	Green	Green	Green
Powder + Nitrocellulose	Green	Green	Green
Powder + 1N NaOH in methanol	Green	Green	Green
Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Green	Dark brown	Brown
Powder + 1N HCl	Brownish green	Black	Dark brown
Powder + 1N HCl + Nitrocellulose in amyl acetate	Green	Black	Reddish brown
Powder + 1N NaOH in water	Green	Black	Brownish green
Powder + 1N NaOH in water, dried and mounted in Nitrocellulose in amyl acetate	Dark green	Black	Brownish green
Powder + HNO_3 (1:1)	Brownish green	Brown	Brown
Powder + H_2SO_4 (1:1)	Dark green	Dark brown	Brownish green

Results for TLC are tabulated in Table - 5.

Color reaction of powdered drug with different reagents and their fluorescence analysis were studied and recorded in Table - 6.

DISCUSSION

The diagnostic microscopic features of leaf are presence of covering unicellular trichomes and anomocytic stomata. The powder microscopy of leaf also showed presence of unicellular trichomes, stomata, spiral thickening and fibers. Presence of covering unicellular trichomes, collenchyma, chlorenchyma, collateral vascular bundle are few of the important characteristics of the petiole and rachis.

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs i.e. presence or absence of foreign inorganic matter such as metallic salts and / or silica (19).

In present study, the stomatal index and number of stomata was found to be 33.6 and 20 respectively. Vein islet number was found to be 17. The total moisture content was found to be 56.18%, along with total ash 12.9 %, of which, 1.5 % is acid insoluble ash, and 4.3 % is water soluble ash. The extractive values were found to be 18.08% and 19.84% for water and alcohol respectively.

Thin Layer Chromatography revealed that methanol gives better extraction of the phytochemicals than water since the methanolic extract resolved into maximum number of bands as compare to aqueous extract.

The pharmacognostical study is one of the major criteria for identification of plant. The present study on the pharamcognostical characteristics of *Cardiospermum halicacabum* L. leaves will provide useful information for its correct identity and may enable those who handle this plant to maintain its quality control. In addition the results of the present study could be useful for preparation of a monograph of the plant.

REFERENCES

1. Kirtikar K.R., Basu B.D. *Indian Medicinal Plants*. 2nd ed. Allahabad, India: Published by Lalit Mohan Basu; 1984.
2. Kumaran A. and Karunakaran R. J. Antioxidant activities of the methanol extract of *Cardiospermum halicacabum*. *Pharmaceutical Biology*. **44**(2): 146–151 (2006).
3. Venkatesh Babu K. C. and Krishnakumar S. *Cardiospermum halicacabum* suppresses the production of TNF-alpha and nitric oxide by human peripheral blood mononucluear cells. *African journal of Biomedical Research*. **9**: 95–99. (2006).
4. Dhar L. M., Dhar M. M., Dharwan N. B., Mehrotra N. B. and Ray C. Screening of Indian Plants for biological activity. *Indian Journal of Experimental Biology*. **6**: 232–247 (1968).
5. Sadique J., Chandra T., Thenmoshi V. and Elango V. Biochemical modes of action of *Cassia occidentalis* and *Cardiospermum halicacabum* in inflammation. *Journal of Ethnopharmacology*. **19**: 201–212 (1987).
6. Raman N., Radha A. and Ravi M. Antibacterial activity of *Cardiospermum halicacabum* against human and plant pathogens. *Indian Drugs*. **35**(1): 29–31 (1998).
7. Gurib Fakim A., Sweraj M.D. Studies on the antisickling properties of exracts of *Sideroxylon puberulum, Faujasiopsos flexuosa, Cardiosper-*

mum halicacabum and *Pelargonium grareolens*. *Planta Med*. **58**: 648–649 (1992).

8. Boonmars T., Khunkitti W. and Sithithaworn P. *In vitro* antiparasitic activity of extracts of *Cardiospermum halicacabum* against third-stage larvae of Strongyloides stercoralis. *Parasitol Research*. **97**: 417–419 (2005)
9. Padmini D.S., Ramya Sravani K.M., Saraswathy G.R., Maheswari E. and Ashok Kumar C.K. Internation Seminar on Medicinal Plants and Herbal Products. Sri Venkatsswara Univ., Tirupati, A.P. Sponsored by NPMB (National Plant Medicinal Board). 2008.
10. Eswar Kumar K., Mastan S.K., Amarender Reddy G., Raghunandan N., Sreekanth N. and Chaitanya G. Anti-arthritic property of the ethanolic leaf extract of *Cardiospermum halicacabum* Linn. *Biomedical and Pharmacology Journal*. (1) **2**. 2008
11. Subramanyam R., Newmaster S. G., Paliyath G. and Newmaster C. B. Exploring ethnobiological classifications for novel alternative medicine: A case study of *Cardiospermum halicacabum* L. (Modakathon, Ballon Vine) as a traditional herb for treating rheumatoid arthritis. *Ethnobotany*. Vol **19**: 1–16 (2007).
12. Mukharjee P. K. *Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals*. India: (Business Horizones; 2005).
13. Khandelwal K.R. *Practical Pharmacognosy: Techniques and Experiments*. 4th ed. India: (Nirali Prakashan; 1998).
14. Kokate C.K., Purohit A.P. and Gokhale S.B. *Pharmacognosy*. 37th ed.(Nirali Prakashan. 2007).
15. Wagner H. and Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. Berlin: (Springer; 1996).
16. Kokoski J., Kokoski R. and Salma F. J. *J. Am. Pharm. Ass.*; **47** Suppl 10: 715–717 (1958).
17. Chase C.R. and Pratt R.J. *J. Am. Pharm. Ass.* **38**: 324–333 (1949).
18. Madhurima, Ansari SH, Alam P, Ahmad S, and Akhtar MS. Pharmacognostic and phytochemical analysis of *Gymnema sylvestre* R.(Br.) leaves. *Journal of Herbal medicine and Toxicology*. **3** (1): 73–80 (2009).
19. Musa K.Y., Katsayal A.U., Ahmed A., Mohammed Z. and Danmalam U.H., Pharmacognostic investigation of the leaves of *Gisekia pharnacioides*. *African J of Biotechnology*. **5** (10), 956–957 (2006).

Alpha-Glucosidase Inhibitory And Hypoglycemic Activities Of *Physalis Minima* Extract.

Sathis Kumar D.*, Raju S.N., Harani A., Banji David, Rao K.N.V., Banji Otilia

Nalanda College of Pharmacy, Nalgonda -508 001, Andhra Pradesh, India.

ABSTRACT

Alpha glucosidase in-vitro inhibitory activity and hypoglycemic effect by oral administration in rats of *Physalis minima* ethanol extracts have been investigated. *Physalis minima* extract showed in-vitro inhibitory activity of intestinal alpha glucosiadase enzyme maltase. Analysis of data confirms that alpha glucosidase inhibition activity was maximum at 1000mcg/ml of *Physalis minima*. The purpose of study was to know whether *Physalis minima* extract could reduce intestinal absorption of monosaccharides by inhibiting disaccharide hydrolysis. The post prandial elevation in blood glucose level at 60 and 120 min after administration of maltose with *Physalis minima* extracts (200 mg/kg and 400 mg/kg doses) showed significant suppression compared to control group. These results suggest that the *Physalis minima* extract has potent alpha glucosidase inhibitory activity and would be effective in suppression of elevation in blood glucose after oral administration of maltose to rats.

Keywords: alpha-glucosidase inhibition, hypoglycemic activity, *Physalis minima*.

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** satmpdina@yahoo.co.in

INTRODUCTION:

Intestinal glucosidase enzymes play an important role in carbohydrate digestion and absorption. Therefore an inhibitor of intestinal glucosidase could be expected to retard carbohydrate digestion and absorption. A reasonable way to control these carbohydrate dependent diseases would be to limit intestinal carbohydrate digestion. It has been recognized that alpha glucosidase inhibitors can be used to prevent some disorders such as diabetes, obesity, hyperlipidaemia and hyperlipoproteinaemia (1) and also show anti-HIV activity (2). It is essential for hyperglycemic conditions that the intestinal absorption of dietary carbohydrates be suppressed by inhibiting intestinal glucosidase, which delay the digestion of oligosaccharides and disaccharides to monosaccharide and reduce the rate of glucose absorption, rise in blood glucose levels and insulin response. Research has recently been conducted on glucosidase inhibitors obtained from plant sources which show reduction in postprandial blood glucose concentrations like onion (3), clove (4), tea (5). A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes, and is more strongly associated with the risk for cardiovascular disease than are fasting glucose levels (6). Potent glucosidase inhibitors such as acarbose and voglibose have already been clinically used for diabetic and obese patients.

Physalis minima belong to Solanaceae family, distributed in South Asian countries. *Physalis minima* is commonly found on the bunds of the fields, waster lands, around the houses, on roadsides, etc., where the soil is porous and rich in organic matter. It is an annual herbaceous plant having a very delicate stem and leaves. The whole plant are bitter, appetizing, tonic, diuretic, laxative, and useful in inflammations, enlargement of the spleen and abdominal troubles (7). Hence, we have conducted this present study to know in vitro inhibitory activity of *Physalis minima* extract on alpha glucosidase enzyme maltase and subsequent in vivo reduction of glucose absorption by inhibiting disaccharide digestion.

MATERIAL AND METHODS

Preparation of plant material *Physalis minima* was collected from local areas of Nalgonda, Andhra Pradesh

and authentified by Mr. Madhavachetty, Botanist, S.V.University, Thirupati, Andhra Pradesh. Plant was dried in the shade and ground into uniform powder using milling machine

Physalis minima (500g) was extracted with 70% ethanol in Soxhlet apparatus for 24 hrs. After filtration and evaporation of ethanol, the residue obtained was 5.8%. The phytochemical screening proved that the plant consists of tannin, polyphenols, alkaloids and polysaccharides.

Male Wistar rats (150-200 g) were fed with a standard diet and water *ad libitum*. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27°C and 12 hours light / dark cycle) throughout the experimental period. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

In vitro alpha glucosidase inhibitory activity:

Physalis minima ethanolic extract was used to investigate the in-vitro inhibitory effect of alpha glucosidase enzymes. After fasting, small intestine of goat between duodenum and cecum(Upper Part) was cut, rinsed with ice-cold saline and homogenated with maleate buffer (pH 6). Small intestine homogenate was used as an enzyme source. The 500 µl of enzyme & 100 µl of extract of different concentration and Acarbose (1000mcg/ml) were taken in to different test tubes and pre incubated for 15min, at 37°C. Then 500 µl of 100 mM maltose(2%) as a substrate was added to all the test tubes and incubated for 15min at room temperature and centrifuged. 0.6ml of supernatant liquid was collected from all the test tubes separately and it was mixed with 0.8ml of alkaline $CuSO_4$ individually The solution was heated in water bath for 8min and cooled. After cooling, phosphomolybdic acid was added to the mixture and made to 10ml with distilled water. Glucose concentration was measured using glucose kit (Accucheck). In case of maltase inhibitory test, maltose was used as a substrate. Table 1: represents the values of invitro studies.

Table 1: In vitro effect of alpha glucosidase inhibitory activity

Concentration (mcg/ml)	***% inhibition activity***	
	Physalis minima	**Standard**
500	34.31±1.259**	
1000	50.99±1.984**	51.78±2.615**
1500	48.08±0.525**	
2000	48.06±1.959**	

The values are mean±SD of 6 values. Means with superscripts (**) within a column are significantly different from each other at $p < 0.01$ as determined by Dennett's Multiple comparison test. F value is 90.41, df (5, 12).

Evaluation of Hypoglycemic activity in Physalis minima fed normal wistar rat.

Normal Wister rats were randomly divided in to 3 groups (6 rats /group) and were fasted overnight (18hrs). Animals in-group I were treated with tragacanth(1%) as control, group II animals were administered with *Physalis minima* extract 200 mg/kg and group III animals were treated with *Physalis minima* 400 mg/kg orally. Blood samples were taken from the lateral tail vein at 0, 60, 120, 180minutes. The blood glucose concentration was measured by using glucometer and noted. The noted values presents in table 2.

Table 2: **Evaluation of hypoglycemic activity in *Physalis minima* fed normal wistar rat**

Group	***I (control)***	***II (200mg/kg)***	***III (400mg/kg)***
Initial	106.33 ± 0.5773	112.33 ± 1.5274**	112.33 ± 1.52**
1 hour	103.33 ± 2.0816	101.33 ± 0.57** (9.792%)	103.66 ± 1.52 (7.718%)
2 hours	107 ± 1	82.66 ± 2.08** (26.413%)	85.33 ± 2.08** (24.036%)
3 hours	106.33 ± 1.157	79.66 ± 1.52** (29.083%)	83.66 ± 0.57** (25.523%)

Values are Mean ± SD,N = 6

**P < 0.01, *P < 0.05 Vs. Control

Figures in parenthesis indicates the percentage decrease in blood glucose level

Evaluation of hypoglycemic activity in Physalis minima fed rat using maltose tolerance test[8]*:*

Normal Wistar rats were randomly divided in to 3 groups. (6 rats/group) and were fasted overnight (18hrs). Animals in-group I were treated tragacanth(1%) along with maltose (2g/kg body weight) as control and the experimental rats are groups II animals were treated with *Physalis minima* extract 200 mg/kg along with maltose (2g/kg body weight)and group III animals treated with *Physalis minima* 400 mg/kg along with maltose (2g/kg body weight). Blood samples were taken from the lateral tail vein at 0, 60, 120, 180minutes. The blood glucose concentration was measured by using glucometer and noted. The noted values presents in table 3.

STATISTICAL ANALYSIS:

All data were subjected to analysis of variance (ANOVA). The data (mean±standard deviation) shown are mean value and the significance differences was compared by using Dennett's Multiple comparison test at the $p < 0.05$ probability level. ANOVA was carried out by using GRACHPADPRISM version 4.2 software.

RESULTS

In vitro alpha glucosidase inhibitory activity:

Graph 1 represents the in vitro effect of alpha glucosidase inhibitory activity shows the inhibitory activity of *Physalis minima* ethanolic extract on maltase in vitro (experiment 1). *Physalis minima* ethanolic extract inhibited alpha glucosidase enzyme in a dose-dependent manner and 1000mcg/ml of *Physalis minima* extract resulted in 50.99% maltase enzymatic inhibitory activity compared with 1000mcg/ml of Acarbose standard.

The evaluation of hypoglycemic activity in Physalis minima fed normal wistar rat

Graph 2 represents the evaluation of hypoglycemic activity in *Physalis minima* fed normal wistar rat shows the changes in the levels of blood glucose in group I control and experimental *Physalis minima* fed groups group II and group III. Group II and III showed suppression of blood glucose elevation at 120 min and 180 min significantly ($p < 0.01$) compared to control. In this study, *Physalis minima* extract significantly ($p < 0.01$) suppressed blood glucose compared with control group during 120min to 180 min period. The blood glucose level of the *Physalis minima* extract administered rats was identical to the level in control group during period from 60 and 120 min. These results showed that *Physalis minima* extract had a suppressive effect on blood glucose after oral administration of extract in rats. Percentage of reduction of blood glucose from the normal level is 29.08% and 25.52% for both 200mg/kg and 400mg/kg of ethanalic *Physalis minima* extract respectively.

Evaluation of hypoglycemic activity in Physalis minima fed rat using maltose tolerance test

Graph 3 represents evaluation of hypoglycemic activity in *Physalis minima* fed rat using maltose tolerance test the shows the changes in the levels of blood glucose in group I control and experimental *Physalis minima* fed group II and group III after oral administration of maltose (2g/ kg). *Physalis minima* treated rat groups showed suppression of blood glucose elevation at 120 min and 180 min significantly ($p < 0.01$) compared to control (maltose) group. In this study, *Physalis minima* extract significantly ($p < 0.01$) suppressed the postprandial elevation in blood glucose compared with control group during 120min to 180 min period after

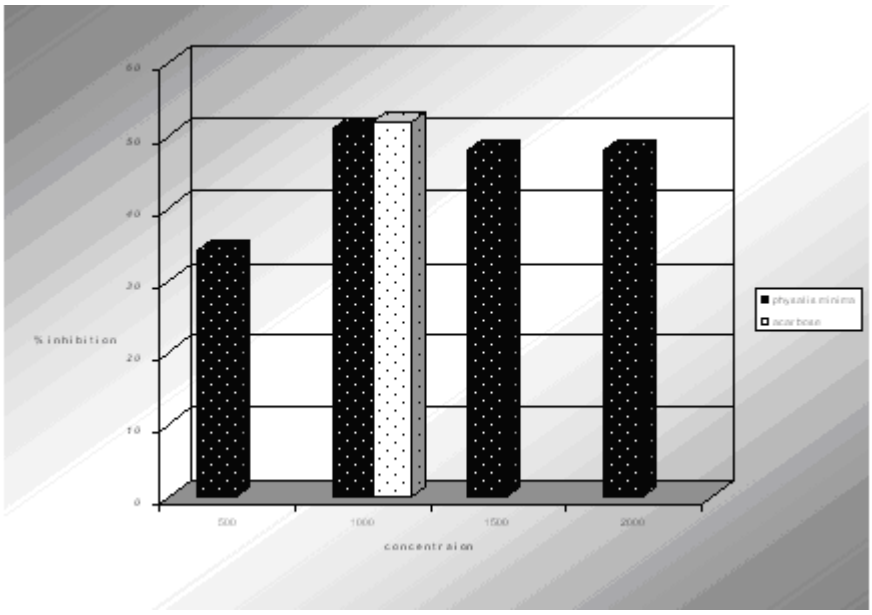
Table 3: Evaluation of hypoglycemic activity in *Physalis minima* fed rat using maltose tolerance test

Group	*I (control)*	*II (200mg/kg)*	*III (400mg/kg)*
Initial	106.33 ± 0.5773	104.66 ± 0.5773	103.66 ± 1.5470*
1 hour	103.33 ± 2.0816	112.33 ± 5.8594	108.33 ± 7.5055
2 hours	107 ± 1	101.67 ± 1.1547 (9.489%)	98 ± 6.0827* (9.53%)
3 hours	106.33 ± 1.157	99.66 ± 6.6583 (11.279%)	97.33 ± 7.2341 (25.523%)

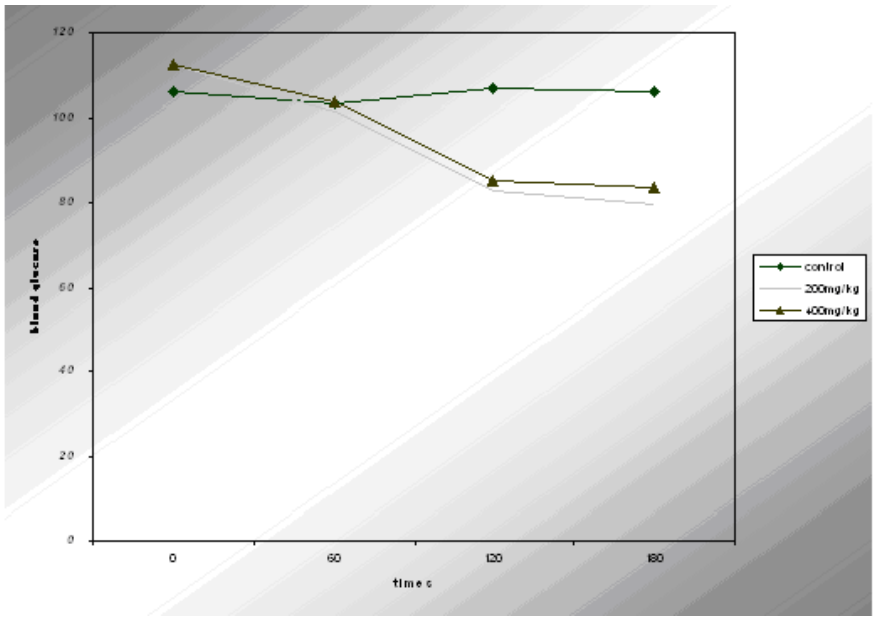
Values are Mean ± SD, N = 6

**P < 0.01, *P < 0.05 VS. Control

Figures in parenthesis indicates the percentage decrease in blood glucose level



Graph 1: In vitro alpha glucosidase inhibitory activity of *Physalis minima*

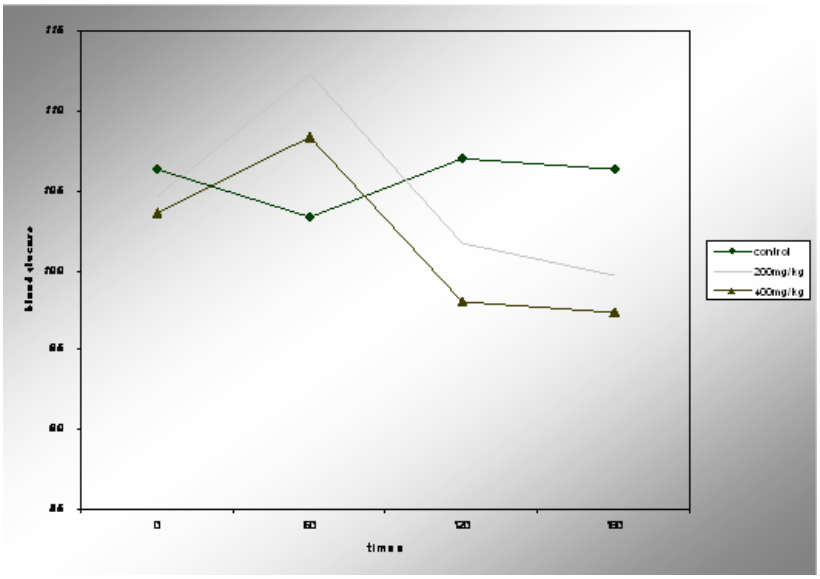


Graph 2: Evaluation of hypoglycemic activity in *Physalis minima* fed normal wistar rat

maltose loading. The blood glucose level in treated rats was identical to the level in control group during period from 60 and 120 min. These results showed that *Physalis minima* extract had a suppressive effect on the post-prandial elevation in blood glucose after maltose oral administration in rats. Percentage of reduction of blood glucose from the elevated level is 11.27% and 25.52% for both 200mg/kg and 400mg/kg of ethanalic *Physalis minima* extract respectively.

DISCUSSION:

This present study shows that ethanol extract of *Physalis minima* had inhibitory activities against maltase that is present in small intestinal mucosa. This is in accordance with recent research conducted on glucosidase inhibitor obtained from plant source and their ability to suppress the postprandial blood glucose level. The methanolic extracts of S. reticulata and S. oblonga strongly inhibited



Graph 3: Evaluation of hypoglycemic activity in *Physalis minima* fed rat using maltose tolerance test

rat intestinal maltase in vitro with IC50 values 42 µg/ml and 32µg/ml respectively, *Physalis minima* were equivalent to the effect of *S. reticulata* and *S. oblonga*. It had been reported that digestive enzymes such as lipase, alpha amylase, and alpha glucosidase, were inhibited by proanthocyanidins and tannins in young chicks, which decreased the digestibility of protein, starch and lipid (9,10). The mechanism of inhibition on maltase intestinal enzymes by ethanolic *Physalis minima* extract could be done to the polyphenolic content. Arecanut extract showed inhibition of elastase and hyaluronidase on skin tissues, which was purified by each fraction of solvents and was identified as a phenolic substance that showed competitive inhibition with the substrate (11). In another study, tea polyphenol such as catechin have been found to inhibit glucosidase activity and glucose transport (12). Tannins (polyphenol) have specific property of precipitating some proteins. This precipitation is presumed to occur by the formation of hydrogen bonds between the hydroxy groups of tannins and the peptide linkages of proteins (13).

Tannins were present in sufficiently high concentrations in *Physalis minima* which might have significantly precipitated the enzyme maltase. In this study, an *Physalis minima* extract was examined for its in vitro inhibition of rat intestinal alpha glucosidase and its in vivo effect on suppression of elevating blood glucose level. *Physalis minima* extract treated group (200mg/kg and 400 mg/kg doses) showed significant suppression ($p < 0.01$) in blood glucose elevation at 120 min and 180 min ($p < 0.01$) compared to maltose loading control rat group. These results suggest that *Physalis minima* extract had a suppressive effect on post prandial elevation in blood glucose after oral administration of maltlose to rats. This study is in accordance with earlier report stated that anthocyanins inhibited alpha glucosidase activity and reduced blood glucose levels after starch rich meals (14). The results strongly suggest that *Physalis minima* extract inhibited blood glucose elevation by inhibiting glucosidase activity, however, it may take part in other mechanism. It is necessary to investigate the mechanism of action of *Physalis minima* extract on glucose transport and insulin secretion. Alpha glucosidase inhibitors are used worldwide for the treatment of diabetes and alpha glucosidase inhibit reversibly the enzymatic cleavage of complex carbohydrates to simple absorbable sugars and hence slow the absorption of carbohydrate from the small intestine, thereby lowering postprandial hyperglycemia.

CONCLUSION:

In conclusion, our findings show that ethanolic *Physalis minima* extract inhibition on maltase may be due to several polyphenolic compounds present within the extract. More studies and in vivo experiments in diabetic conditions are required to ascertain the compounds and its mechanism of action, thereby providing a natural hyperglycemic control treatment, and thus decrease risk for diabetes, cardiovascular diseases. However, further studies are needed before *Physalis minima* polyphenol can be used safely as food additives and supplements.

ACKNOWLEDGEMENT:

The authors are grateful to the Managament, Nalanda College of pharmacy for providing support to carry out this work.

REFERENCES:

1. Puls W, Keup U, Krause HP, Glucosidase Inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. *Naturwissen-schaften* **64**: 536 537. (1977).
2. Ratner L, Glucosidase inhibitors for treatment of HIV-1 infection. *AIDS Res. Hum. Retroviruses*, **8**(2): 165–173 (1992).
3. Nisioka T, Watanabe J, Kawabata J, Niki R, Isolation and activity of N-coumaroyl tyramine an glucosidase inhibitor in welsh onion (Allium Fistulosum). *Biosci. Biotechnol. Biochem*, **61**: 1138–1141. (1997).
4. Toda H, Kawabata J, Kasuari T, Alpha-glucosidase Inhibitors From Clove(Syzgium Armactum). *Biosci.Biotechnol.Biochem.* **64**: 294–298 (2000).
5. Honda M, Hara V, Inhibition of rat small intestinal sucrase and glucosidase activities by tea polyphenols. *Biosci. Biotechnol. Biochem*, **57**: 123–124. (1993).
6. Del Prato S. Post-prandial hyperglycaemia-the necessity for better control, *Int J Clin Pract Suppl.* **112**: 3S–8S. (2000).
7. Warrier PK, Nambiar VPK, Ramankutty C, *Indian Medicinal plants, compendium of 500 species*, vol-**4**, (Orient Longman private limited, Chennai, India, 2006).
8. Senthil Amudhan M, Hazeena Begum V. Alpha-glucosidase inhibitory and hypoglycemic activities of Areca catechu extract. *Phcog Mag.* **4**(15): 223–227 (2008).
9. Yuste P, Longstaff M, McCorquodale C. The effect of proanthocyanidin-rich hulls and proanthocyanidin extracts from bean (Vicia faba L.) hulls on nutrient digestibility and digestive enzyme activities in young chicks. *Br. J. Nutr.* **67**: 57–65. (1992).
10. Longstaff M, McNab JM, The inhibitory effects of hull polysaccharides and tannins of field beans (Vicia faba L.) on the digestion of amino acids, starch and lipid and on digestive enzyme activities in young chicks. *Br. J. Nutr.* **65**: 199–216. (1991).
11. Kuk.-Kook Lee, Cho JJ, Park J, Choi JD, Anti-elastase and anti-hyaluronidase of phenolic substance from Areca catechu as a new anti-ageing agent. *International Journal of Cosmetic Science*. **23**(6): 341–346 (2001).
12. Kobayashi Y, Suzuki M, Hideo S, Arai S, Yukihiko H, Suzuki K, Miyamoto Y, Shimizu M, Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a competitive mechanism. *J. Agric. Food Chem* **48**: 5618–5623. (2000).
13. Loomis WD, Battaile J, Plant phenolics and the isolation of plant enzymes. *Phytochemistry*, **5**: 423. (1966).
14. McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibits alpha-amylase and alpha-glucosidase. *J. Agric. Food Chem.*, **53**: 2760–2766. (2005).

In vitro evaluation of anthelmintic activity of anterdhum padhati mashi and bahirdhum padhati mashi of green *Cocos nucifera* Linn. (Palmae) husk.

Baheti A M*[1], Khandelwal K. R.[2], Baheti Shikha A.[3]

[1]MAEER's Maharashtra Institute of Pharmacy, Paud Road, Pune, India- 411038.
[2]J.S.P.M S Rajarshi Shahu College of Pharmacy and Research, Tathawde, pune – 33
[3]Nabira Mahavidyala, Katol

ABSTRACT

The aim of present study was to evaluate anthelmintic potential of anterdhum Padhati Mahi (APM) and Bahirdhum Padhati mashi (BPM) using Pheretima posthuma and Ascardia galli as test worms. Various concentrations (10 – 100 mg/ml) of APM and BPM were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control. The results of present study indicated that APM and BPM significantly demonstrated paralysis, and also caused death of worms especially at higher concentration of 100 mg/ml, as compared to standard reference Piperazine citrate. In conclusion, the Mashi as an anthelmintic have been confirmed.

Keywords: *Ascardia galli, Kalpravriksha, Pheretima posthuma,* Piperazine citrate,.
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** Mobile No. +91-09422060118 ; Email: akshaybaheti@rediffmail.com

INTRODUCTION

Cocos nucifera Linn. (Family: Palmae, English: Coconut Palm) is extensively cultivated in southern India and Ceylon. Every Part of the tree is being used for some purpose like food, fuel or timber hence it is called as kalpravriksha (1). Recent results show that aqueous extracts from husk of *Cocos nucifera* present antimicrobial, antiviral (2) and antileishmanial properties. The coconut milk was tested for antiparasitic activity in mice and showed efficacy against *Syphacia obvelata*, *Aspiculuris tetraptera* (3) and *Vampirolepis nana* (4). The liquid extracted from the coconut husk fiber has analgesic , antioxidant (5) and antiproliferative activity against lymphocytes (6). By chromatographic methods coupled to mass spectroscopy techniques has been demonstrated that drug contain catechin and epicatechin together with condensed tannins (2). Coconut husk mashi has antimicrobial, diuretic and analgesic activity. Parasitic diseases caused by helminthes lead to significant health hazards to animals resulting in enormous economic impact. While a number of anthelmintics are currently available, all are encountering resistance. Mashi shows presence of tannins and tannins shows anthelmintic activities (7).The objective of this work was to evaluate in vitro the anthelmintic activity of anterdhum padhati mashi (APM) and bahirdhum padhati mashi (BPM) obtained from green coconut husk fiber against *Pheretima posthuma* and *Ascardia galli*.

MATERIAL AND METHODS

Plant material

Cocos nucifera Linn. husk was collected by A. M. Baheti from Pune region and identified by Pharmacognosy Department of MAEER's Maharashtra Institute of Pharmacy, Pune. [Voucher specimen (hp/Cocos/01)]

Preparation of the anterdhum padhati mashi (APM)

Husk was packed in between two earthen pots (Sharav samput), which were sealed by Multtani matti. It was subjected to Gajaputa (heating into kund filled with cow dung cake) in Gajaputa kund for 50 min. When Gajaputa became swangsheet (cool), sharav was taken out of kund and Mashi was collected. 100 gm of the husk gave 31.89 gm of APM Mashi

Preparation of the bahirdhum padhati mashi (BPM)

Husk was collected and dried under shade. Green coconut husk were heated in earthen pot at 145-1550C. With continuous stirring till the white fumes ceases to come out. 100 gm of the husk gave 6gm of BPM Mashi (The burnt black powder).

Worms collection

Indian earthworm *Pheretima posthuma* of about 5-7 cm long were collected from logged area of soil, Adawad, Dist: Jalgaon (MS) and *Ascardia galli* (Nematode) worms were obtained from freshly slaughtered fowls (*Gallus gallus*).

Preparation of test sample

Samples for in-vitro study were prepared by dissolving 2.5 gm of each Mashi in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 50 and 100 mg/ml.

Anthelmintic assay

The anthelmintic assay was carried as per the method of Ajaiyeoba E.O. et al (8) with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (9-12). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro (13-16). *Ascardia galli* worms are easily and plentily available from freshly slaughtered fowls and its use, as a suitable model for screening of anthelmintic drug was advocated earlier (17-19). 50 ml formulations containing three different concentrations, each of mashi (10, 50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both types of worm. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

RESULTS AND DISCUSSION

As shown in Table 1, anterdhum padhati mashi (APM) and bahirdhum padhati mashi (BPM) exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for both types of worms. The BPM caused paralysis of 15 min and time of death of 34 min while

Table 1: Anthelmintic activity of anterdhum padhati mashi (APM) and bahirdhum padhati mashi (BPM)

Sample	Concentration mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute			
		P. posthuma		*A. galli*	
		P	D	P	D
Piperazine citrate	10	24 ± 0.6	56 ± 0.4	17 ± 0.6	31 ± 0.7
APM	10	28 ± 0.5	57 ± 0.7	21 ± 0.2	41 ± 0.6
APM	50	23 ± 0.6	39 ± 0.8	13 ± 0.3	36 ± 0.4
APM	100	17 ± 0.3	40 ± 0.2	14 ± 0.1	24 ± 0.3
BPM	10	26 ± 0.3	58 ± 0.6	22 ± 0.5	40 ± 0.4
BPM	50	20 ± 0.8	45 ± 0.2	18 ± 0.3	32 ± 0.1
BPM	100	15 ± 0.7	34 ± 0.5	11 ± 0.2	21 ± 0.6

All values represent Mean ± SEM; n=6 in each group. Control worms were alive up to 24 hrs of observation.

APM revealed paralysis of 17 min. and time of death of 40 min. respectively against the earthworm *P. posthuma*. The reference drug Piperazine citrate showed the same at 24 and 56min, respectively.

Ascardia galli worms also showed sensitivity to the APM and BPM significantly higher concentration of 100 mg/ml. The BPM caused paralysis at 11 min. and time of death of 21 min. APM showed paralysis at 14 min and the time of death were 24 min. Piperazine citrate exhibited similar effects at 17 and 31 min. respectively. BPM and APM shows presence of tannins which are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g.niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (20).

It is possible that tannins contained in the mashi produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (21) or glycoprotein on the cuticle of the parasite (22) and may cause death.

CONCLUSION

Mashi showed anthelmintic activity. BPM showed good *invitro* anthelmintic activity than APM.

REFERENCES

1. Bose T.K., *Fruits of Indian Tropical and subtropical*, (Naya Prakashan, Calcutta, 1985).
2. Esquenazi D., Wigg M.D., Miranda M.M., Rodrigues H.M., Toster J.B., Rozental S., da Silva A.J. and Alviano C.S. Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. *Research in Microbiology*. **153**: 647–652 (2002).
3. Amorim A. and Borba H.R. Ação anti-helmíntica de plantas X Testes "in vivo" com extratos brutos de Cocos nucifera L. (Palmae). *Rev. Bras.Farm*. **75**: 91–92 (1994).
4. Amorim A. and Borba H.R. Ação anti-helmíntica de plantas XI Influência de extratos brutos de Cocos nucifera L. (Palmae) na eliminaçãodeVampirolepis nana em camundongos. *Rev. Bras. Farm*. **76**: 98–99 (1995).
5. Alviano D.S., Rodrigues K.F., Leitào S.G., Rodrigues M.L., Matheus M.E., Fernandes P.D., Antoniolli A.R. and Alviano C.S. Antinociceptiveand free radical scavenging activities of Cocos nucifera L. (Palmae) husk fiber aqueous extract. *J. Ethnopharmacol*. **92**: 269–273 (2004).
6. Kirszberg C., Esquenazi D., Alviano C.S. and Rumjaneka V.M. The effect of a catechin-rich extract of Cocos nucifera on lymphocytes proliferation. *Phytother. Res*. **17**: 1054–1058 (2003).
7. Niezen H., Waghorn G.C., Charleston W.A.G. and Waghorn G.C. Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (Medicago sativa) or sulla (Hedysarum coronarium), which contains condensed tannins. *J. Agri. Sci*. **125**: 281–89 (1995).
8. Ajaiyeoba E.O., Onocha P.A. and Olarenwaju O.T. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. *Pharm. Biol*. **39**: 217–20 (2001).
9. Chatterjee K.D. *Parasitology, Protozoology and Helminthology*, (Guha Ray Sree Saraswaty Press Ltd., Calcutta, 1967) 168–169.
10. Vidyarthi R.D. *A Text Book of Zoology*, (S.Chand and Co., New Delhi, 1967)
11. Thorn G.W., Adams R.D., Braunwald E., Isselbacher K.J. and Petersdorf R.G. *Harrison's Principles of Internal Medicine*, (McGraw Hill Co., New York, 1977) 1088.
12. Vigar Z. *Atlas of Medical Parasitology*, (P.G.Publishing House, Singapore, 1984)
13. Sollmann T. Anthelmintics: Their efficiency as tested on earthworms. *J.Pharmacol.Exp.Ther.* **12**: 129–170 (1918).
14. Ganapaty Dash G.K., Suresh P., Sahu S.K., Kar D. M. S. and Panda S.B. Evaluation of Evolvulus alsinoids Linn. for anthelmintic and antimicrobial activities. *J.Nat.Rem*. **2**: 182–185 (2002).
15. Shivkar Y.M., Kumar V.L. Anthelmintic activity of latex of Calotropis procera. *Pharm Biol*. **41**: 263–265 (2003).
16. Szewezuk V.D., Mongelli E.R. and Pomillo A.B. Antiparasitic activity of Melia azadirach growing in Argentina. *Mole. Med. Chem.* **1**: 54–57 (2003).
17. Kaushik R.K., Katiyar J.C. and Sen A.B. Studies on the mode of action of anthelmintics with Ascardia galli as a test parasite. *Ind. J. Med. Res*. **62**: 1367–1375 (1974).
18. Lal J., Chandra S., Raviprakash V. and Sabir M. In vitro anthelmintic action of some indigenous medicinal plants on Ascardia galli worms. *Ind. J. Physiol. Pharmacol*. **20**: 64–68 (1976).
19. Kumar D., Mishra S.K., Tandan S.K. and Tripathi H.C. Possible mechanism of anthelmintic action of palsonin on Ascardia galli. *Ind. J.Pharmacol*. **27**: 161–66 (1995).
20. Martin R.J. Mode of action of anthelmintic drugs. *Vet J*. **154**: 11–34 (1997).
21. Athnasiadou S., Kyriazakis F., Jackson R.L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vitro and in vivo studies. *Vet. Parasitol*. **99**: 205–219 (2001).
22. Thompson D.P. and Geary T.G. The structure and function of helminth surfaces. In: Marr, J.J. eds. *Biochemistry and Molecular Biology of Parasites*. (Academic Press, New York, 1995) 203–232.

A Survey of Medicinal Weed Flora of Wheat (*Triticum aestivum* L. em.Thell.) Fields in Mahendergarh District of Haryana (India)

Kumar Pawan[1], Yadav Sanjay[2], Gollen Babita[1], and Yadava R. K.[1*]

[1]Department of Genetics, CCS Haryana Agricultural University,
Hisar (Haryana) Email: pk5gene@rediffmail.com
[2]Department of Genetics, Maharishi Dayanand University, Rohtak, India
[1*]Department of Genetics, CCS Haryana Agricultural University,
Hisar (Haryana); Email: rameshyadav53@yahoo.com

ABSTRACT

A total of 19 species of weeds in wheat fields representing 11 families were collected from the different region of Mahendergarh district of Haryana during the winter seasons of November 2007 to April 2008 and November 2008 to April 2009. The survey revealed that a significant number of weed species has an ethnomedicinal value, besides this some weeds are highly nutritious and their leaves may be used as food or herbal medicine. The study suggested that there is a tremendous scope of utilizing medicinal weed plants to provide a supplementary income to farmers.

Keywords: Ethnomedicine, Weed, Traditional knowledge, Wheat,.
Editor: Mueen Ahmed KK, Phcog.Net

INTRODUCTION

From the beginning of human civilization, human beings depend on plant resources especially for the food and medicinal purpose. People throughout the world use medicinal plants and have great faith on them for their effectiveness due to their inherent medicinal properties. Relatively expensive allopathic treatment may further lead people, both of rural and urban areas to depend on the herbal medicines (1). Practice of pharmacogonosy in India goes back to the Vedic period. The Rigveda claims about 99 medicinal plants, The Yajurveda 82 and the Atharveda 28, which were used to cure lots of deadly diseases. In Samveda too, a number of medicinal plants has been mentioned (2). Ayurveda being the study of several drugs and their uses is the foundation stone of the ancient medicinal science of life and art of healing. Charaka Samhita and Susrata Samhita are great ancient Indian ethics relating to the treatment of various diseases and ailments that reflect a glorious period of phytotherapy (2, 3).

Wheat is a major staple food crop of India facing problems by a lot of weeds infestation (4). The plant species cannot be considered as weed under all circumstances. In the present study, it has been restricted to weeds infesting the wheat fields of the district Mahendergarh, Haryana (India). Weeds of wheat field, regarded undesired and neglected as the constant sources of annoyance and trouble to the farmers are simply eradicated by plucking and throwing away; but in fact, they are important from the standpoint of medicinal and food values. (5). A number of weeds are edible and their leaves may be used for food or herbal medicine for curing different diseases of humans & cattles. Some researchers have also reported medicinal industrial and allelopathic uses of several obnoxious weeds (5–9). The southern region of Haryana offers immense scopes for medicinal weed plant research as a numerous species are used against various disease ailments by local peoples, in different area of the region (4, 5).

The study area (Mahendergarh) is located at south Haryana near the border of Rajasthan. The district lies between north latitude 27°47' to 28° 26' and east longitude 75° 56' to 76° 51'. It covers an area of 1899 Km^2. and comprises 4.3 percent of total area of Haryana. Most of the population lives in the rural areas and engaged in agriculture. In Mahendergarh farmers do not emphasize on chemical weed control. They only perform hand

weeding operations at intervals. As the problems of labor scarcity and high wages are increasing in the region, hand weeding is becoming a costly operation. The most important positive aspect of the weeds is that nearly all of them are known to possess therapeutic properties and are used by the native people for cure of a variety of human and cattle diseases. By identifying the medicinal, industrial and allopathic uses of existing weed flora, a package can be developed for the farmers. By selling the useful weed parts farmers can not only recover the cost of hand weeding and control the weed population efficiently but also can earn additional income (9). In Mahendergarh), various wild herbs have been collected and sold in the local market by local communities. However, very limited scientific knowledge is available on the potential herbs, which can be cultivated and utilized for different purposes. Survey is done in order to list out the existing weed flora and their medicinal, industrial and allopathic uses.

MATERIAL AND METHODS

The ethnobotanical survey was conducted in whole district during November 2007-April 2009 by using questionnaire method. A total 22 villages across the district are surveyed. The villages covered in the survey were Budhwal, Sahbajpur, Nagal Choudhary, Megot-halla, Shima, Kanwi, Shimli, Manndi, Nizampur, Rambas, Dharson, Hudina, Pali, Jerpur, Satnali, Bawana, Sehlang, Bhagot, Dahina, Bhojawas, Ateli and Kanina (Fig. 1) of district Mahendergarh (Table 2). The road map of Haryana state was followed and the routs planned to establish sampling localities as the complete district covered. From each selected village random sampling was done during various field visits and plant identification was done by spot identification method with the help of flora. Diverse age group people ranging from 25-105 years and vaids of that area were interviewed for collection of the ethnomedicinal uses of common weed species growing in wheat crop fields in the study area. Beside this the help of reference literatures of Ayurveda, Homoeopathy, Yunani, and other systems of medicine was also taken for knowing the medicinal uses of the weeds.

Table 2 List of villages covered in the survey.

Sr.No.	Symbol	Village Name
1	A	Budhwal.
2	B	Sahbajpur.
3	C	Nagal Choudhary.
4	D	Megot halla.
5	E	Akbarpur.
6	F	Kanwi.
7	G	Manndi.
8	H	Nizampur.
9	I	Rambas.
10	J	Dharson.
11	K	Hudina.
12	L	Pali.
13	M	Jerpur.
14	N	Bawana.
15	O	Sehlang.
16	P	Bhagot.
17	Q	Dahina.
18	R	Bhojawas.
19	S	Kanina.
20	T	Ateli.

RESULTS AND DISCUSSION

The results of the present study shows that weeds found in wheat fields of Mahindragarh district plays an important role in the livelihood of the people. Agriculture is the main employment of India and 80% population of study area is busy in agriculture. Wheat is the major staple food of Mahindragarh district of Haryana. A total of 20 different villages across the district are covered in the survey. Nineteen different weeds were reported from the area belonging to eleven families. Out of these thirteen plant species possesses valuable medicinal properties. The medicinal properties of these 13 weeds have been found well documented in reference literatures. Beside medicinal use few plants are used as fodder both in fresh and dry form. The Local name, scientific name, Family and traditional uses of these medicinal weeds are given in Table 1. Most of the weeds have medicinal values and are locally used for various diseases. Due to modernization young people migrates to urban areas and only old people possess the knowledge about plant uses in treatment of various problems. Young generation is more depends on Allopathic drugs for the treatment of various problems as they provide quick relief as compared to Ayurveda but Ayurvedic medicines have very little side effects as compared to Allopathic medicines (10–14).

The plants were classified as shrubs and herbs. Based on the utility, there were 19 medicinal plants, 2 species are pot herbs and 13 fodder plants. The personal communications were made with more than 30 national and international drug dealers revealed that these weeds were having heavy demands in national and international drug markets.

During the study it was noted that different graded and processed parts or weeds were having more demand.

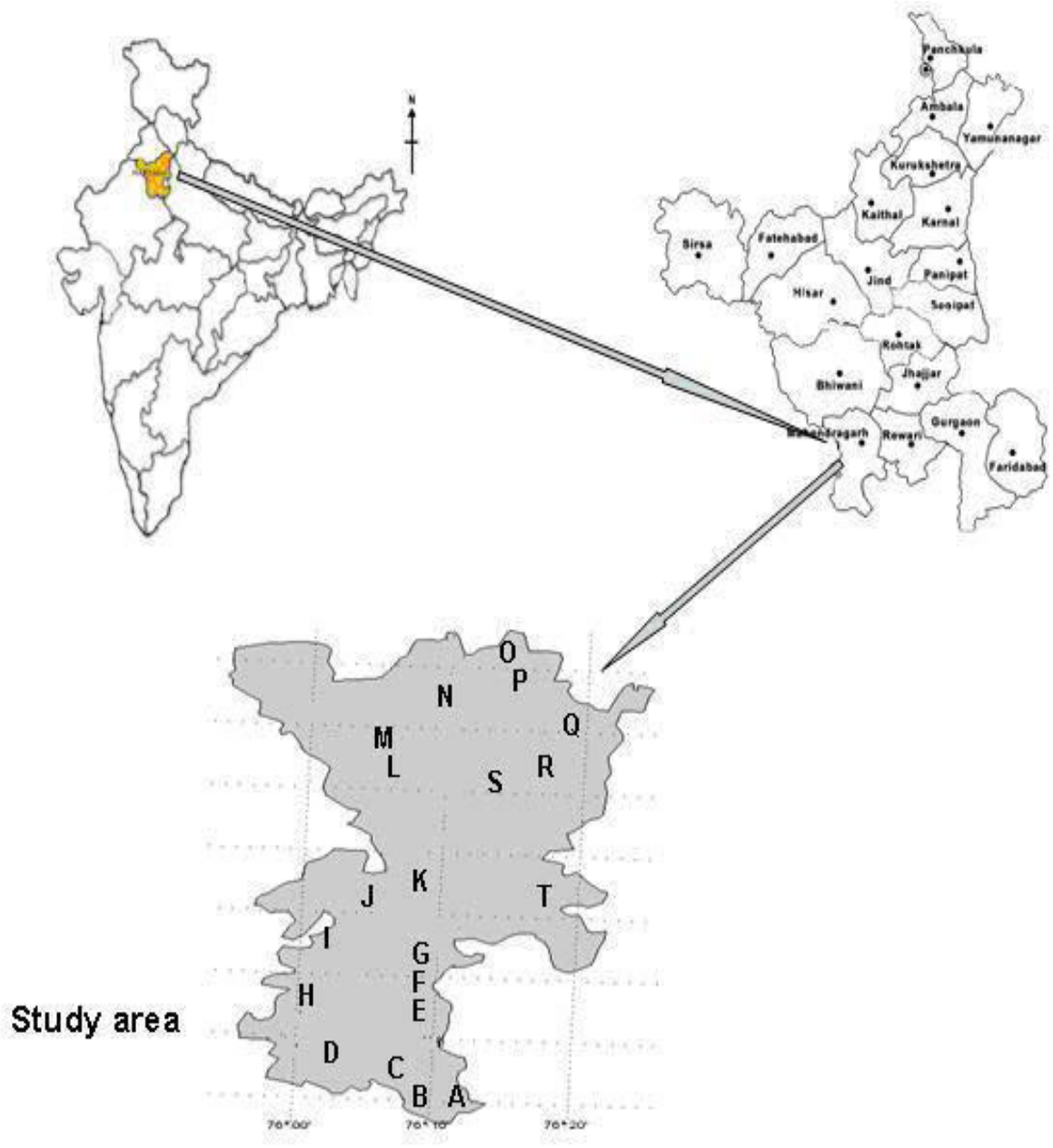


Figure 1 Location of villages

There is a tremendous scope of generating employment opportunities at village level for rural youths by this medicinal weed business (9). The study suggested that there is a strong need of

Documentation of valuable knowledge about medicinal weeds of wheat fields.

To locate the areas having higher density of medicinal weeds.

Establishment of proper linkage between village farmers and national and international drug dealers.

Development of grading and processing methods for medicinal weeds according to international standards.

These targets can be achieved by the joint efforts of government, non-government agencies and local people.

ACKNOWLEDGEMENT

Authors are thankful to Dr. J.P. Yadav, Prof., Department of Genetics, Maharishi Dayanand University, Rohtak,

Table - 1. List of weeds and their medicinal uses

Sr.No.	Botanical Name	Family	Flowering	English Name	Local Name	Parts Used	Preparations	Traditional Uses	Medicinal Uses
1	*Anagallis arvensis* L.	Primulaceae	Jan- March	Scarlet Pimpernel	Billi Buti	Whole plant	Paste, Decoction	As fodder, To expel leaches from the cattle	Used to cure leprosy, diuretic, purgative, and for curing hydrophobia.
2	*Argemone mexicana* L	Papoveraceae	February-March	Yellow Mexican Poppy	Satyanasi	Leaves, Seeds, Stem	Juice of shoot	As manure	Used to treat malarial fevers, jaundice, leprosy and skin diseases.
3	*Asphodelus tenuifolius* Cav.	Asphodelaceae	March-April	Wild onion	Piazi	Whole plant.	Paste, Juice	As manure	As diuretic, anti-inflammatory and prevent atherosclerosis.
4	*Avena ludoviciana* Dur	Poaceae	January-February	Wild oats	Jangli Jai	Whole plant	Paste	As fodder	Used in inflammation.
5	*Carthamus oxyacantha* Beib.	Asteraceae	April	Yellow Thistle	Pholi	Stem, Roots	Decoction	As fodder	Oil is obtained which is used in heart diseases.
6	*Chenopodium album* Sturm	Chenopodiaceae	January-February	Lambsquarters	Bathua	Whole plant	Plant paste, juice,decoction	As food & fodder,	Highly nutritious, Sag prepared and given to anaemic patients, antithelminthic & laxative properties.
7	*Chenopodium murale* L	Chenopodiaceae	January-February	Neetle- leaved Goosefoot	Khartua	Leaves & Seeds.	Paste	Green dyes is obtained from the plant	Used to treatment of sterility, digestive problems, hair loos and cough.
8	*Chichorium intybus* Linn.	Asteraceae	April	Chicory	Kashni	Whole plant	Paste	As fodder	Useful in headache, asthma and prevent bilious vomiting.
9	*Cirsium arvense* L Scop	Asteraceae	March	Canada thistle	Kateli	Fruits & Roots.	Decoction	As food	Diuretic, astringent, antiphlogistic and hepatic. Decoction is used to treat worms in children.

Sr.No.	Botanical Name	Family	Flowering	English Name	Local Name	Parts Used	Preparations	Traditional Uses	Medicinal Uses
10	*Convolvulus arvensis* L.	Convolvulaceae	March-April	Field bindweed	Hiran khuri	Stem & roots	Powder, Juice	As fodder.	Roots are purgative and possess Blood coagulating properties, Diuretic; Laxative.
11	*Cynodondactylon* (L.) Pers	Poaceae	March	Bermuda Grass	Dubh	Whole plant	Decoction	As fodder.	Plant juice is diuretic, used in dropsy, diarrhea and dysentery.
12	*Fumaria indica* (Hausskn.) Pugsley	Fumariceae	April	Fumitory	Papri	Whole plant	Decoction	As fodder.	Used in constipation, diarrhea, antispasmodic, diaphoretic, diuretic and laxative tonic.
13	*Lolium temulentum* L.	Poaceae	April	Rye Grass	Rye Ghas	Seed.	Paste	Food additive.	Anti inflammatory and used in the treatment of cancer,
14	*Melilotus indica* L.	Fabaceae	March	Not known	Senji	Whole plant	Crushed	As Green manure	Seeds are used in bowel complaints and skin disease.
15	*Phalaris minor* Retz.	Poaceae	March	Canary grass	Sitti/Gulid-anda	Whole plant	Decoction	As fodder	Not known
16	*Siline cnoidea* L.	Caryophyllaceae	February-March	Forked Catchfly	Takla	Whole plant	Paste, decoction	As fodder	It is used in baths or as a fumigant and in the treatment of ophthalmia.
17	*Sisymbrium irio* L.	Cruciferae	February-March	Wild Mustard	Jangli sarson	Flowers, Leaves & Seed.	Cooking Powder raw	As fodder	Immature leaves are Stimulant and also used in the treatment of asthma, throat affections and sore eyes .
18	*Trigonella polycerata* L.	Fabaceae	March	Not known.	Maini	Leaves & Seeds.	Cooked, powdered	As fodder	Used as antiinflammatory, antiphlogistic, laxative and Parasiticide.
19	*Vicia sativa* L	Fabaceae	Feb-March	Fetch	Rewari/ Rari	Leaves, Seed.	Cooked powder, paste	As fodder, manure.	Paste applied in snake bite.

India, for his valuable help in identification of weed species of the study area. Authors also acknowledge the help received from the villagers during the field study.

REFERENCES

Maiti R.K., Petriz-Olvera R.A., Sanchez-Arreola E., Osvaldo Ramirez E. and Singh V.P. Medicinal plants species selected and confirmed for their efficiency to cure different diseases. *Crop Res*, **25**(3): 550 (2003).

1. Bhattacharya A.K. and Patra K. Medicinal plants for rural women and child health care-issues, options and strategy. *Indian For*, **130**(4): 385 (2004).
2. Oudhia P. and Tripathy R.S. Scope of cultivation of important medicinal plants in Chhattisgarh plains. *Proc. Nat. Conf. Healthcare Dev. Herbal Medicines, Raipur*. **215** (1999).
3. Malik R.K., Pawar R.S., Katyal S.K. and Bhan V.M. Weed seed distribution in wheat-A case study. *Haryana J. Agron.* **1**: 17–20 (1985)
4. Oudhia P. and Dixit A. Weeds in Ambikarpur region (Madhya Pradesh) and their traditional use. *Weed News* **1**(2): 1–5 (1994).
5. Chopra R.N., Nayar S.L. and Chopra I.C., *Glossary of Indian Medicinal Plants*, (CSIR, New Delhi, 1956).
6. Prusti A.B. and Panda J. Some wild plants as food items used by the Tribal people of Sundargarh district, Orissa. *Adivasi*. **45**(2): 30–38 (2005).
7. Memon M.I.A. and Shahani N.M. *Survey and domestication of wild medicinal plants distribution in Sindh province of Pakistan*. (Annual Research Report. Dept. of PBG Sindh Agric. Uni. Tandojam. 191–193 1986).
8. Hassan G. and Marwat K.B. Integrated weed management in agricultural crops. *National workshop technologies for Sustainable Agriculture*. (NIAB, Faisalabad 24–26 2001).
9. Ibrar M.S., Hashim A. and Marwat K.B. Ethnobotanic study of the weeds of five crops in District Abbottabad, *N-W. Pak. J. Weed Sci. Res.* **9** (3&4): 229–240 (2003).
10. Shad R.A. Rabi Major and Weeds Kharif of Agronomic Crops of District Bannu. Status of Weed Science activities in Pakistan. *Progressive Farming*. **7**(1): 10–16 (1987).
11. Das P.K. and Mishra M.K. Some medicinal plants used by the tribal of Deomali and adjacentareas of Koraput District, Orissa. *Indian J.For.* **10**(4): 301–303 (1987).
12. Murty K.S, Padhi M.M., Chopra K.K. Some plants used as anti-fertility agents by 'Khonds' of Orissa. *Bulletin of Medico-EthnoBotanical Research*. **18**: 164–166 (1997)
13. Yadav S., Arya V., Kumar S. and Yadav J.P. Plants of Haryana useful in dermatological disorders: An Ethnobotanical Survey. *Phcog Rev.* **3**(5): 94–97 (2009).

Antioxidant Activities of Methanolic and Aqueous Extracts from Leaves of *Martynia annua* Linn.

Nagda Dhruti[1]*, Saluja Ajay[1], Nagda Chirag[2]

[1]A. R. College of Pharmacy & G. H. Patel Institute of Pharmacy,
Vallabh Vidyanagar-388120, Gujarat, India.
[2]Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar-388121, Gujarat, India.

ABSTRACT

The antioxidant activity of the methanolic and aqueous extracts of *Martynia annua* Linn. leaves were evaluated by several *in vitro* systems of assay, namely, reducing power assay, DPPH radical-scavenging activity, nitric oxide scavenging activity, H_2O_2 radical scavenging activity, superoxide radical scavenging assay, hydroxyl radical-scavenging activity, and total antioxidant capacity. Total phenolic content was measured by Folin–Ciocalteau reagent. The antioxidant property depends upon concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extract. The results showed that the methanolic extract exhibited higher antioxidant activity than the aqueous extract. Chlorogenic acid is reported in this plant and a TLC densitometric method was developed for the quantification of Chlorogenic acid.

Keywords: *Martynia annua* Linn., antioxidant, chlorogenic acid, HPTLC.
Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** dhruti_nagda@yahoo.com

INTRODUCTION

Active (or reactive) oxygen species and free radical-mediated reactions have been implicated in degenerative or pathological processes such as aging, cancer, coronary heart disease and Alzheimer's disease (1). Meanwhile many epidemiological results point to an association between a diet rich in fresh fruit and vegetable and a decrease in the risk of cardiovascular diseases and certain forms of cancer in humans (2). Several reports concern the antioxidant activities of natural compounds in fruits and vegetables. These include phenolic compounds (3), anthocyanin (4), water extracts of roasted *Cassia tora* (5).

In cells, metabolic pathways normally couple to degrade free radicals. If the generation rates of free radicals are faster than degradation rates under environmental stresses, cells suffer oxidative stresses. Two distinct pathways, nonenzymatic or enzymatic, were found in plant cells as routes of free radical scavengers. The former included ascorbate or chlorogenic acids (6) or vitamin E; the latter included different forms of SOD to metabolize superoxide free radical to hydrogen peroxide. The hydrogen peroxide produced was further metabolized either by catalase or different forms of peroxidase such as glutathione peroxidase (7).

The genus *Martynia* comprises of 7 species native to Mexico. *Martynia annua* Linn., a native of Mexico belongs to the Martyniaceae family and common names include Devil's Claw (English), Bichhu (Hindi), Kakanasika (Sanskrit) and Vichchida (Gujarati). The leaves and fruits are biologically active part of this plant (8, 9). The leaves of the *Martynia annua* are edible and used as antiepileptic and antiseptic, applied locally to tuberculous glands of the neck; the juice of the leaves as a gargle for sore throat and the leaf paste for wounds of domestic animals (10, 11). Some important chemical constituents of *M. annua* include phenolic acids like chlorogenic acid, sinapic acid, p- hydroxybenzoic acid; Several flavonoids including apigenin, luteolin, apigenin-7-O-beta- D- glucuronide (12, 13).

In the present paper, we report our work on establishing antioxidant activity of *M. annua* leaves, in quenching free radicals that are generated in several *in vitro* models. We also report the presence and TLC densitometric quantification method of chlorogenic acid, in order to evaluate a relationship between the antioxidant activity

and the phytochemical constituents of the *M. annua* leaves.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts of the plant were collected between the months of August-September 2008, from Anand district of Gujarat State, India. The collected plant was authenticated by a Taxonomist, Bioscience Department, Sardar Patel University, Vallabh Vidyanagar. The Voucher specimen of *Martynia annua* was deposited under DCP/Ma-2/23 in A.R.College of Pharmacy, Vallabh Vidyanagar. After collection, the leaves were washed thrice with water to remove dust and debris. The washed leaves were dried under shade and powdered (60#). The powdered leaves of *Martynia annua* (100 g) were soaked in petroleum ether (600 mL) and allowed to stand for 48 h, with occasional shaking. The macerate was decanted and filtered, through cloth, and then, through Whatman filter paper. The residue, called 'marc,' after the extraction with petroleum ether was dried and extracted exhaustively with methanol to yield a greenish brown semisolid (11.68 % W/W). The same procedure was followed for preparation of aqueous extract by taking chloroform water instead of methanol to yield a reddish brown solid (22.32 %W/W). The methanol extract of *Martynia annua* (MEMA) and aqueous extract of *Martynia annua* (AEMA) were used for evaluation of antioxidant activity.

Determination of total polyphenols

The Total phenolic content in the MEMA and AEMA extracts were determined with the Folin-Ciocalteau reagent according to the method of Chandler and Dodds (14) using gallic acid as a standard phenolic compound. Briefly, 1 mL of extract solution (1000 µg/mL) in a volumetric flask diluted with distilled water (46 mL). FC reagent (1 mL) was added and the contents of the flask were mixed thoroughly. After 3 minutes, 3 mL of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The amount of total phenolic compounds in the plant extract was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard gallic acid graph:

$$y = 0.0047\times - 0.0879,\ R^2 = 0.9798$$

Reducing Power Assay

The reducing power of MEMA and AEMA extracts were determined by the method of Oyaizu (15). The capacity of extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. For this purpose, different concentrations of plant extract (100–1000 µg/mL) in 1 ml of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes Aliquots (2.5 mL) of Trichloro acetic acid (TCA, 10%) were added to the mixture. The 2.5 mL of solution was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm by spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing ability.

DPPH free radical scavenging activity

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (16). 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (10–200 µg/mL). It was incubated at room temperature for 45 minutes and the absorbance was measured at 517 nm against corresponding blank solution. Ascorbic acid was taken as reference. Percentage inhibition of DPPH free radical was calculated based on the control reading, which contained DPPH and distilled water without any extract using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_{cont} - A_{test}) / A_{cont} \times 100$$

Where A_{cont} was the absorbance of the control reaction and A_{test} was the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction (17). 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 150 minutes. From the incubated mixture 0.5mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 minutes. Finally,

1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 minutes. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100]$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract/Standard.

Hydrogen peroxide scavenging activity

H_2O_2 scavenging ability of extract was determined according to the method of Ruch et al. (18). A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Extract at the different concentrations (10–1000 µg/mL) in 3.4 mL phosphate buffer were added to a H_2O_2 solution (0.6 mL, 40 mM). The absorbance of the solution was measured at 230 nm against a blank solution containing the extract without H_2O_2. The percentage of H_2O_2 scavenging of extract and standard compounds was calculated as:

$$\% \text{ Scavenged} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ was the absorbance of the control, and A_{sample} was the absorbance in the presence of the sample of extract.

Superoxide radical scavenging activity

The scavenging activity of the MEMA and AEMA extracts towards superoxide anion radicals were measured by the method of Liu, Ooi, and Chang (19). 100 µL riboflavin solution [20 µg], 200 µL EDTA solution [12 mM], 200 µL methanol and 100 µL NBT (Nitro-blue tetrazolium) solution [0.1 mg] were mixed in test tube and reaction mixture was diluted up to 3 mL with phosphate buffer [50 mM]. The absorbance of solution was measured at 590 nm using phosphate buffer as blank after illumination for 5 minutes. This is taken as control. Different concentrations of extracts were taken, to each of this, 100 µL riboflavin, 200 µL EDTA, 200 µL methanol and 100 µL NBT was mixed in test tubes and further diluted up to 3 mL with phosphate buffer. Absorbance was measured after illumination for 5 minutes at 590 nm on UV visible spectrophotometer.

Hydroxyl radical (OH–) scavenging activity

The scavenging ability for hydroxyl radical was measured by Zhao et al. method (20). Stock solution of EDTA (1mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM) H_2O_2 (10 mM) and deoxyribose (10 mM) was prepared in distilled de- ionized water. The attempt was performed by adding up 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.1 mL H_2O_2, 0.36 mL deoxyribose, 1 mL of sample extract (100–500 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50mM, pH 7.4) and 0.1mL of ascorbic acid added. The mixture was incubated at 37°C for 1 h. A 1.0 mL of incubated mixture was mixed with 1.0 mL of 10% trichloro acetic acid and 1.0 mL of 0.5% thiobarbituric acid (in 0.025M NaOH containing 0.025% BHA) to urbanized the pink color measured at 532 nm. The hydroxyl radical scavenging activity is reported as percent inhibition of deoxyribose sugar dilapidation and is calculated as

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of control and A_{sample} is the absorbance of test.

Determination of Total antioxidant capacity

Total antioxidant capacity was measured according to the method reported by Prieto with slight modifications (21). In brief, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 mL of alcohol, combined separately in an eppendroff tube with 1.9 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 minutes. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg per mg of extract.

Quantification of marker compounds by HPTLC

TLC Conditions

Plate: Precoated HPTLC aluminum sheet (MERCK) Silica gel $60F_{254}$ (10cm×10cm)

Spotter: Camag Linomat V, a sample applicator as bands using the spray-on technique, Camag Switzerland.

Developing chamber: CAMAG glass twin trough chamber (20 × 10 cm)

Scanner: CAMAG TLC Scanner 3 and WINCATS software (version 4.06)

Experimental conditions: Temperature 37°C, relative humidity 40 %

Solvent system: Ethyl acetate: Formic acid: Methanol (9:2.4:1.5)

Preparation of sample solution

5.0 g of leaf powder of *M. annua* was extracted exhaustively with methanol (4 × 100 ml) and the extract was filtered, pooled and the combined extract was concentrated under vacuum and volume was made up to 25 ml in a volumetric flask.

Preparation of standard solutions

Standard solution of chlorogenic acid: Accurately weighed 10mg standard Chlorogenic acid was taken in 10 ml volumetric flask. Add 10ml of methanol and dissolved. (1mg/ml)

Calibration curve

Calibration curve for chlorogenic acid: Graded concentration of standard solution of Chlorogenic acid (solution A, 1mg/ml=1000 ng/µl) in which 1, 2, 3, 4, 5 and 6 µl volumes were applied on a precoated HPTLC silica gel 60 F_{254} (10×10cm) using Camag Linomat V automatic spotter. The concentrations of Chlorogenic acid were 1000, 2000, 3000, 4000, 5000 and 6000 ng/ spot.

Quantification of chlorogenic acid in the leaves of M. annua

10 µl of the sample solution was applied in triplicate on a pre-coated silica gel 60 F_{254} HPTLC plate. The plate was developed in the solvent system of Ethyl acetate: Formic acid: Methanol (9:2.4:1.5) and scanned at 329nm. The peak areas and absorption spectra were recorded. The amount of chlorogenic acid in the sample was calculated using the calibration curve.

Method validation

ICH guidelines (CPMP/ICH/381/95; CPMP/ICH/281/95) were followed for the validation of the analytical procedure. The method was validated for precision, repeatability and accuracy. Instrumental precision was checked by repeated scanning of the same spot of chlorogenic acid seven times and was expressed as coefficient of variance (% CV). The repeatability of the method was affirmed by analyzing 3000 ng/spot of chlorogenic acid after application on the TLC plate (n = 6) and was expressed as % CV. Accuracy of the method was tested by performing recovery studies at three levels (50%, 100% and 150% addition). The percent recovery as well as average percent recovery was calculated. For the evaluation of limit of detection and limit of quantification different dilutions of the standard solution of chlorogenic acid were applied along with methanol as blank and determined on the basis of signal to noise ratio.

RESULTS AND DISCUSSION

Total Polyphenols

Total Phenolic Content (TPC) was determined in comparison with standard gallic acid and the results expressed in terms of µg GAE/g dry sample. The TPC values for MEMA (42.53 ± 0.71) and AEMA (31.89 ± 0.86), all as µg Gallic Acid Equivalent (GAE)/g dry sample, respectively. As phenolics are responsible for antioxidant activity, generally, it is expected that extract/drug which contains high TPC would show highest total antioxidant capacity.

Reducing power assay

MEMA and AEMA had effective reducing power using the potassium ferricyanide reduction method when compared to the standard (Ascorbic acid) (Table 1). For the measurement of the reductive ability of MEMA and AEMA, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of MEMA and AEMA using the method of Oyaizu. At different concentrations MEMA (r^2 = 0.9852) and AEMA (r^2 = 0.9928) demonstrated powerful reducing ability. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The reducing power increased as the MEMA concentration increased, indicating some compounds in *M. annua* is both electron donors and could react with free radicals to convert them into more stable products and to terminate radical chain reactions when compared with AEMA. The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity.

DPPH free radical scavenging activity

Table 2 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of MEMA, AEMA and ascorbic acid (r^2= 0.8916). The scavenging effect of MEMA (r^2= 0.9524), AEMA (r^2= 0.9237) and ascorbic acid on the DPPH radical decreased in the order of ascorbic acid > MEMA > AEMA, which were 79, 57 and

Table 1: Reducing power ability of MEMA, AEMA and ascorbic acid

Concentration (µg/ml)	MEMA	AEMA	Ascorbic acid
100	0.394 ± 0.0023	0.120 ± 0.0031	0.340 ± 0.0025
200	0.654 ± 0.0035	0.189 ± 0.0016	0.620 ± 0.0103
400	1.151 ± 0.0041	0.301 ± 0.0027	0.780 ± 0.0032
600	1.546 ± 0.0118	0.446 ± 0.0024	0.991 ± 0.0019
800	1.884 ± 0.0021	0.599 ± 0.0013	1.201 ± 0.0118
1000	2.135 ± 0.0032	0.787 ± 0.0009	1.435 ± 0.0025

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

Table 2: Free radical scavenging activity of MEMA, AEMA and ascorbic acid in DPPH method

Concentration (µg/ml)	% free radical scavenging activity of MEMA	% free radical scavenging activity of AEMA	% free radical scavenging activity of Ascorbic acid
10	13.26 ± 1.45	6.51 ± 1.22	39.77 ± 0.86
20	20.91 ± 0.86	13.37 ± 1.73	49.37 ± 1.63
40	26.97 ± 2.17	19.77 ± 0.66	57.14 ± 1.22
60	33.83 ± 1.39	21.25 ± 1.43	65.49 ± 0.92
80	42.97 ± 0.84	30.63 ± 1.79	71.66 ± 1.18
100	57.83 ± 0.92	42.63 ± 2.64	79.89 ± 0.80
200	77.03 ± 1.74	54.51 ± 1.73	92.46 ± 1.29

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

Table 3: Nitric oxide scavenging activity of MEMA, AEMA and ascorbic acid

Concentration (µg/ml)	% radical scavenging activity of MEMA	% radical scavenging activity of AEMA	% radical scavenging activity of Ascorbic acid
100	29.87 ± 1.22	12.33 ± 0.91	38.18 ± 1.74
200	35.66 ± 0.75	18.62 ± 1.23	43.27 ± 2.18
400	39.43 ± 0.92	34.09 ± 2.78	49.37 ± 0.80
600	45.59 ± 2.37	39.69 ± 1.22	53.65 ± 1.67
800	55.84 ± 1.62	46.54 ± 0.87	62.70 ± 2.14
1000	66.10 ± 1.48	52.01 ± 0.21	72.77 ± 1.21

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

42 %, at the concentration of 100 µg mL^{1}, respectively. DPPH free radical scavenging activity of MEMA and AEMA also increased with increasing concentration. IC_{50} values (concentration of sample required to scavenge 50% free radical) were found to be 105.56 µg/mL, 164.56 µg/mL and 15.81 µg/mL for MEMA, AEMA and ascorbic acid (standard) respectively. In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. This method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radial form DPPH-H by the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (22).

Nitric oxide scavenging activity

The fractions of *M. annua* effectively reduced the generation of nitric oxide from sodium nitroprusside. A MEMA, AEMA and ascorbic acid (standard) exhibited 66.10%, 49.56% and 72.77% inhibition, respectively and the IC_{50} values were found to be 636.44 µg/ml, 912.34 µg/ml and 425.28 µg/ml for MEMA, AEMA and ascorbic acid (standard) respectively (Table 3). Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent (23). The absorbance of the chromophore was measured at 546 nm in the presence of the fractions. All the fractions of *M. annua* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the fractions which compete with oxygen to react with NO^- thereby inhibiting the generation of nitrite.

Hydrogen peroxide scavenging activity

A 1000 µg/ml of MEMA, AEMA and ascorbic acid (standard) exhibited 78.45%, 70.02% and 84.74% inhibition, respectively and the IC_{50} values were found to be 357.69 µg/mL, 422.65 µg/mL and 199.82 µg/mL for MEMA, AEMA and ascorbic acid (std.) respectively as shown in Table 4. The composition of hydrogen peroxide into water may occur according to the antioxidant compounds. Since antioxidant compound/(s) present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O (24).

Superoxide radical scavenging activity

The Table 5 shows concentration dependent scavenging of superoxide radicals by methanolic and aqueous extracts of *Martynia annua*. A 1000 µg/ml of MEMA, AEMA and ascorbic acid (standard) exhibited 72.81%, 62.47% and 84.65% inhibition, respectively and the IC_{50} values were found to be 256.29 µg/mL, 589.58 µg/mL and 50.41 µg/mL for MEMA, AEMA and ascorbic acid (standard) respectively. Superoxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to the oxidative stress (22). The capacity of both the extracts (MEMA and AEMA) to scavenge superoxide radical revealed that these extracts possess superoxide dismutase like activity. Although the activity was found to be lower than scavenging activity of ascorbic acid in all concentration ranges (200 – 1000 µg/mL).

Hydroxyl radical (OH–) scavenging activity

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. All the fractions of *M. annua* and the ascorbic acid (standard) inhibited the production of hydroxyl radicals. A 500 µg/ml of MEMA, AEMA and ascorbic acid exhibited 67.25%, 52.40% and 75.55% inhibition respectively and the IC_{50} values were found to be 309.03 µg/ml, 471.22 µg/ml and 206.59 µg/ml for MEMA, AEMA and ascorbic acid respectively (Table 6). A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. The oxygen derived hydroxyl radicals along

Table 4: H_2O_2 radical scavenging activity of MEMA, AEMA and ascorbic acid

Concentration (µg/ml)	% radical scavenging activity of MEMA	% radical scavenging activity of AEMA	% radical scavenging activity of Ascorbic acid
10	15.41 ± 1.36	18.02 ± 1.07	28.83 ± 1.65
20	23.01 ± 3.17	23.77 ± 2.46	33.05 ± 0.95
40	28.07 ± 0.83	32.44 ± 1.28	38.57 ± 1.73
80	34.59 ± 1.29	38.57 ± 1.76	43.48 ± 1.97
100	46.17 ± 1.75	41.64 ± 0.68	54.53 ± 2.44
200	58.67 ± 1.44	51.61 ± 3.27	62.27 ± 3.57
400	62.19 ± 2.10	55.60 ± 2.64	67.71 ± 0.85
800	72.47 ± 1.68	66.95 ± 1.21	79.68 ± 1.08
1000	78.45 ± 0.68	70.02 ± 2.25	84.74 ± 1.33

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

Table 5: Superoxide radical scavenging activity of MEMA, AEMA and ascorbic acid

Concentration (µg/ml)	% radical scavenging activity of MEMA	% radical scavenging activity of AEMA	% radical scavenging activity of Ascorbic acid
200	47.55 ± 1.28	36.78 ± 0.85	55.12 ± 1.47
400	55.22 ± 0.94	45.42 ± 1.67	63.43 ± 1.56
600	61.09 ± 2.68	50.32 ± 1.44	69.93 ± 1.40
800	66.10 ± 3.33	56.61 ± 3.12	77.93 ± 0.88
1000	72.81 ± 2.10	62.47 ± 1.75	84.65 ± 1.23

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

Table 6: Hydroxyl radical scavenging activity of MEMA, AEMA and ascorbic acid

Concentration (µg/ml)	% radical scavenging activity of MEMA	% radical scavenging activity of AEMA	% radical scavenging activity of Ascorbic acid
100	34.93 ± 0.58	15.72 ± 2.10	41.05 ± 1.14
200	39.74 ± 1.75	21.83 ± 3.66	48.03 ± 1.89
300	47.16 ± 2.10	33.19 ± 2.85	59.83 ± 2.17
400	57.21 ± 1.43	44.54 ± 1.70	66.38 ± 1.55
500	67.25 ± 1.46	52.40 ± 0.82	75.55 ± 1.31

Values are mean ± S.E.M (n=3)

MEMA- Methanolic extract of *Martynia annua;* AEMA- Aqueous extract of *Martynia annua*

with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid (25). All the fractions of *M. annua* when added to the reaction mixture scavenged the hydroxyl radicals and prevented the degradation of deoxyribose.

Determination of Total antioxidant capacity

Total antioxidant capacity of each of methanolic and aqueous extract of *Martynia annua* Linn. is expressed as number of equivalents of ascorbic acid. One mg of methanolic and aqueous extracts contained 240.0 µg and 94 µg of ascorbic acid equivalents antioxidant capacity respectively. The assay was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (26).

Quantification of marker compounds by HPTLC

In the TLC densitometric method, it was observed that the best resolution of chlorogenic acid, avoiding interference from the other components of the extracts, was obtained in the solvent system of Ethyl acetate: Formic acid: Methanol (9:2.4:1.5). The identity of chlorogenic acid in the extracts was established by comparing the Rf (0.68) with the standard compound (Figure 1). HPTLC fluroscent image under the excitation wavelength 329 nm

Table 7: Method validation parameters for the quantification of Chlorogenic acid

Parameters	Chlorogenic acid
Specificity	Specific
Linear range (ng/spot)	1000 – 6000
Correlation coefficient (r^2)	0.9966
Linear regression	y = 6.1012x + 455.34
LOD (ng/spot)	203.21
LOQ (ng/spot)	615.78
% Recovery	96.75 – 99.36
Repeatability (RSD, n=6)	0.715%
Precision (% CV) Intraday (n=3) Interday (n=3)	0.66 – 1.55 0.82 – 1.40
Amount of Chlorogenic acid found in *Martynia annua* leaves (%w/w)	0.100 ± 0.0062

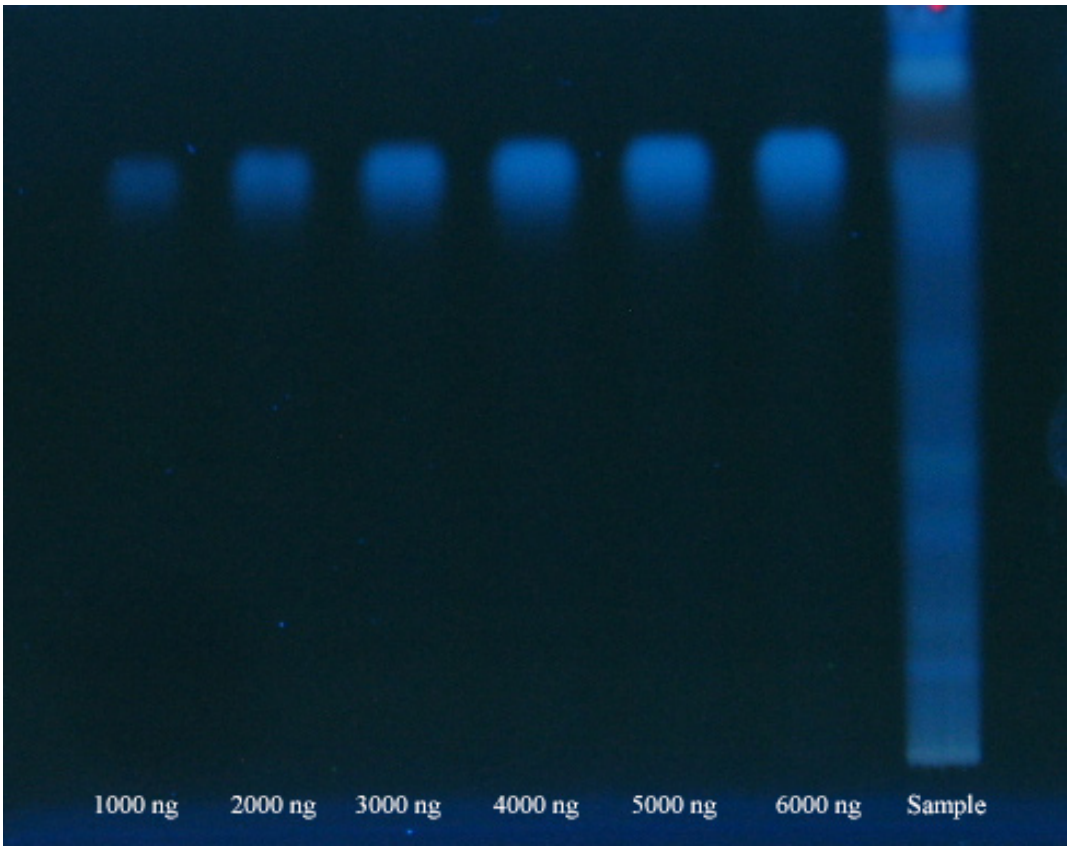


Figure 2. HPTLC fluroscent image under the excitation wavelength 329 nm of standard and sample extract containing Chlorogenic acid.

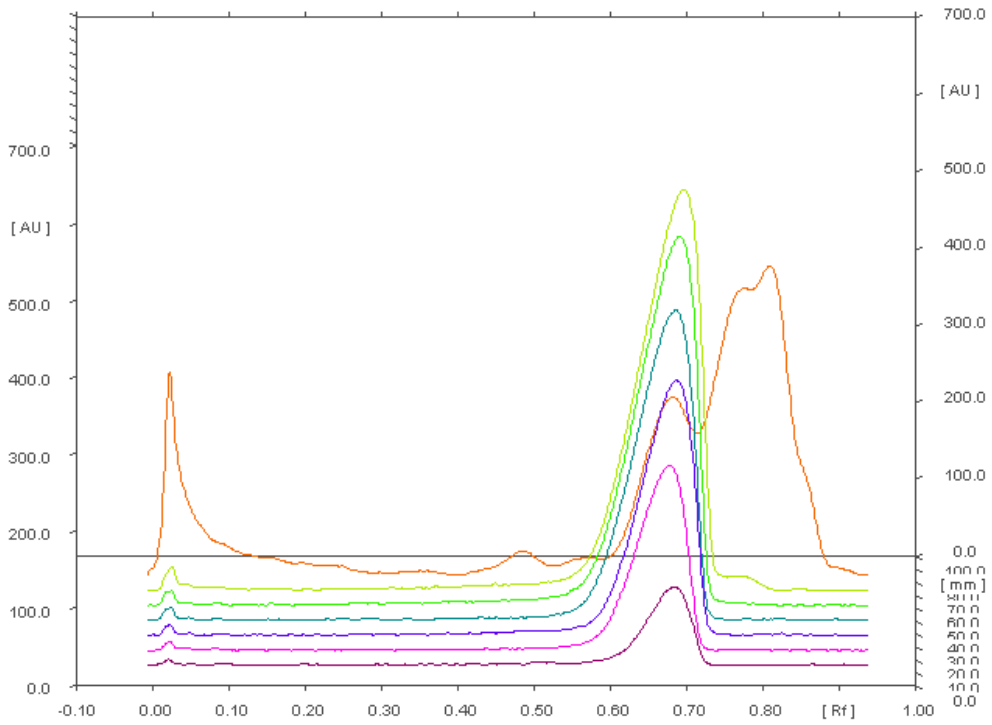


Figure 1. TLC densitometric chromatograms of extract of *Martynia annua* leaves with chlorogenic acid standards.

Table 8: Recovery study of Chlorogenic acid by HPTLC method

Marker compound	Amount present in the sample (ng)	Amount added (ng)	Amount found (ng)	Recovery (%)	Average recovery (%)
Chlorogenic acid	1000	500	1457 ± 74.18	97.13 ± 4.94	97.75
	1000	1000	1935.33 ± 15.57	96.75 ± 0.77	
	1000	1500	2484 ± 48.49	99.36 ± 1.93	

Values are mean ± SD (n=3)

of standard and sample extract containing chlorogenic acid was displayed in Figure 2. Linear relationship was obtained for chlorogenic acid in the range of 1000 – 6000 ng/spot with a correlation coefficient of 0.9966. The method was validated in terms of precision, repeatability and accuracy (Table 7). The limit of detection and limit of quantification were found to be 203.21 ng/spot and 615.78 ng/spot respectively. The content of chlorogenic acid estimated in the *M. annua* leaves extract was found to be 0.100 ± 0.0062 % W/W.

From the recovery studies that were carried out at three different levels i.e. 50 % addition, 100 % addition and 150 % addition of marker compound, the average percentage recovery obtained was 97.75 % (Table 8). Chlorogenic acid is reported for the first time in this plant. It was confirmed by TLC with a standard chlorogenic acid sample. The TLC densitometric method developed for the quantification of chlorogenic acid from *M. annua* leaves was found to be simple precise, accurate and robust and can be used for quality control of *M. annua* leaves.

CONCLUSION

In conclusion, the present study has demonstrated that *Martynia annua* methanolic and aqueous extracts are rich in phenolics and have a strong antioxidant activity and a radical-scavenging action in all of the tested methods. This suggests that *Martynia annua* leaf is a good source of natural antioxidants. The isolation, purification and mechanism action of chlorogenic acid and other components of *M. annua* are of interest for further investigation and will be carried out in future studies.

ACKNOWLEDGEMENTS

The authors are grateful to Director, SICART, Vallabh Vidyanagar, Anand for providing necessary facilities.

REFERENCES:

1. Ames B.N. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*. **221**: 1256–1264 (1983).
2. Salah N., Miller N.J., Paganga G., Tijburg L., Biolwell G.P. and Rice-Evans C. Polyphenolic flavonols as scavenger of aqueous phase radicals and as chain breaking antioxidants. *Arch. Biochem. Biophys*. **322**: 339–346 (1995).
3. Rice-Evans C.A., Miller N.J. and Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci*. **2**: 152–159 (1997).
4. Espin J.C., Soler-Rivas C., Wichers H.J. and Viguera-Garcia C. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. *J. Agric. Food Chem*. **48**: 1588–1592 (2000).
5. Yen G.C. and Chuang D.Y. Antioxidant properties of water extracts from *Cassia tora* L. in relation to the degree of roasting. *J. Agric. Food Chem*. **48**: 2760–2765 (2000).
6. Kono Y., Kashine S., Yoneyama T., Sakamoto Y., Matsui Y. and Shibata H. Iron chelation by chlorogenic acid as a natural antioxidant. *Biosci. Biotechnol. Biochem*. **62**: 22–27 (1998).
7. Hou W.C., Wu W. C., Yang C. Y., Chen H. J., Liu S. Y. and Lin Y. W. Antioxidant activities of methanolic and hot-water extracts from leaves of three cultivars of Mai-Men-Dong (*Liriope spicata* L.). *Bot. Bull. Acad. Sin*. **45**: 285–290 (2004).
8. Chopra R. N., Nayar S. L., Chopra I. C., *Glossary of Indian Medicinal Plants*, (National Institute of Science Communication, New Delhi, 1996) 162.
9. Satyavati G.V., Gupta A.K., Tandon N., *Medicinal Plants of India*, Volume **II**, (Indian Council of Medical Research, New Delhi, 1987) 223–229.
10. Anonymous. *The Ayurvedic Pharmacopoeia of India*. Part I, Volume **III**, (Government of India, Ministry of Health & Family Welfare, New Delhi) 77–78.
11. Anonymous. *The Wealth of India*. Publication and information Directorate (CSIR, New Delhi, 1985) 307.
12. Das V.S.R., Rao K.N. and Rao J.V.S. Phenolic acid in some members of Pedaliaceae. *Current Science*. **35**: 160 (1966).
13. Gunasegaran R. and Sree vidya H. Chemical investigation of the flavonoids of *Martynia annua*. *Fitoterapia*. **63**(1): 88–89 (1992).
14. Chandler S.F. and Dodds J.H. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell Reports*. **2**: 1005–110 (1993).
15. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Jap. J. Nutr.* **44**: 307–315 (1986).
16. Burits M. and Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res*. **14**: 323–28 (2000).
17. Sreejayan N. and Roa M.N.A. Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol*. **49**: 105–107 (1997).
18. Ruch R.J., Cheng S.J. and Klaunig J.E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen*. **10**: 1003–8 (1989).
19. Liu F., Ooi V. E. C. and Chang S. T. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci*. **60**: 763–771 (1997).
20. Yu W., Zhao Y. and Shu B. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. *Food Chem*. **86**: 525–529 (2004).

21. Prieto P., Pineda M. and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, **269**: 337–341 (1999).
22. Kalola J., and Shah M. Free Radical Scavenging activity of *Inula cappa*. *Ars. Pharm.* **47**(4): 385–401 (2006).
23. Marcocci P.L., Sckaki A. and Albert G.M. Antioxidant action of *Ginkgo biloba* extracts EGP761. *Methods Enzymol.* **234**: 462–475 (1994).
24. Ruch R.T., Cheng S.J. and Klaunig J.E. Spin trapping of superoxide and hydroxyl radicals. *Methods Enzymol.* **105**: 198–209 (1984).
25. Umamaheshwari M. and Chatterjee T. *In vitro* antioxidant activities of the fractions of *coccinia grandis* L. Leaf extract. *Afr. J. Trad.* **5**(1): 61–73 (2000).
26. Kubola J. and Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. *Food Chemistry*. **110**: 881–890 (2008).

Antifungal and Antiaflatoxigenic activity of *Aegle marmelos* Linn.

Patil R. H.*[a], Chaudhary Bhushan[b] and Settipalli Sailaxmi[c]

[a, b, c, *]Department of Microbiology and Biotechnology, R.C.Patel Arts, Commerce and Science College, Shirpur 425405 (MS), India

ABSTRACT

Aegle marmelos Linn. (Rutaceae) is reported to possess a number of medicinal properties including antidiarrhoeal, antimicrobial, antifungal and activities. The antiaflatoxigenic effects of ethanolic extract of the leaves of Aegle marmelos were studied on common aflatoxigenic fungal species. Aegle marmelos exhibited antifungal and antiaflatoxigenic activity at a concentration range of 0.5 to 2 mg/ml. The shake flask method was used to evaluate the antifungal and antiaflatoxigenic activity. The extract showed varied levels of antifungal and antiaflatoxigenic activity against the test fungi. Preliminary phytochemical tests of ethanolic extracts demonstrated the presence of major phytochemicals like phenols, tannins, flavonoids and alkaloids as major constituents.

Keywords: Antifungal activity, Aegle marmelos, Antiaflatoxegenic, Synergistic effect

Editor: Patil R. H., Phcog.Net

Corresponding author: E-mail – ravi_nmu@yahoo.co.in

INTRODUCTION

The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial and antiaflatoxigenic effects of natural compounds (1). Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural products. They are toxic to humans and animals, cause significant reductions in crop yield and cause economic losses (2-3). Their occurrence in various countries has been well documented (4). When the fungi invade and grow in commodities such as peanuts, corn and the resulting contamination with aflatoxins often makes the commodities unfit for consumption. Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, mycotoxicosis, sometimes resulting in death (5). Consumption of maize is an important source of aflatoxin, aflatoxin B1 (AFB1) is a highly toxic and carcinogenic metabolite produced by Aspergillus species on food and agricultural commodities (6, 7). In addition, many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against Aspergillus parasiticus (8). Many spices and herbs, such as cloves, anise and star anise seeds, basil, cinnamon, marigold and spearmint, garlic, onion thyme, cassia and sweet basil have been reported to inhibit toxigenic and food borne molds (9). Aflatoxins are known to be potent mutagenic, carcinogenic, teratogenic, hepatotoxic, immunosuppressive and also inhibit several metabolic activities (10). These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (11). Among all classes of aflatoxin, aflatoxin B1 (AFB1) is known to be the most significant in terms of animal and human health risk (12). Thus foods contaminated with these toxigenic fungi and presence of aflatoxin is a major concern, which has received worldwide attention due to their deleterious effects on human and animal health as well as their importance international food trade (13). Although there are many synthetic fungicides in use, their safety in foods are yet to be fully established and also the chances of obtaining newer fungicides that meet stringent environmental and food safety requirements through empirical synthesis do not appear to be high. The use of numerous plant extracts, spices, and their constituents may provide an alternative way to prevent fungal growth and aflatoxin formation (14). Therefore there has been a considerable interest by the food industry and a growing trend in consumer preferences for natural antifungals over synthetic compounds. The present report evaluates the antifungal and antiaflatoxigenic potential Aegle marmelos.

Aegle marmelos Linn (Rutaceae), AM, commonly known as “Bael tree”, is a well known deciduous tree, 7-8

m in height with trifoliate aromatic leaves and bisexual flowers, indigenous to India, Burma and Srilanka, often planted in the vicinity of Shiva temples. It grows wild all over the sub Himalayan forests, central India, its west coast and in dry hilly places. Fresh half ripe fruit is mild astringent, and used to cure dysentery, diarrhea, hepatitis, tuberculosis, dyspepsia and is good for heart and brain. The roots have antidiarrhoeic, antidote to snake venom, anti-inflammatory and wound healing properties. The leaves and seed oil have antifungal antiaflatoxigenic properties (15). In view of above information and folk lore use of roots and fruits of this plant as antidiarrhoeal and anti microbial agent, present study was undertaken to evaluate the antifungal and antiaflatoxigenic activity of ethanol extract of Aegle marmelos Linn.

MATERIAL AND METHODS-

Plant materials and extraction.

The specimens of AM dried leaves were obtained from Toranmal forest, of Nandurbar district , Maharashtra, and authenticated by Dr. D.A. Patil, at the Pharmacognosy department of R.C. Patel College of Pharmacy and the herbarium specimen (RP-094) was prepared and deposited in the same department. The dried leaves of AM were coarsely powdered by using mixer. The powdered drug was passed through sievesofno 40 and powdered pass through the sieve was used for extraction. The extracts obtained were filtered twice and were concentrated under vacuum using rotary vacuum evaporator, (Butchi, Switzerland) and stored in desiccator until use. About 500 mg of AM leaves powder was extracted overnight by cold maceration in ethanol and water (7:3). The yield of ethanol water extract was 8.5 gm.

Test Microorganisms

Cultures were isolated from infected groundnut, maize by using spread plate technique and named as F1, F2, F3, and F4 respectively. The isolated strains were checked for aflatoxin production. The standard aflatoxigenic strains Aspergillus flavus, Aspergillus parasiticus were procured from NCIM, Pune and maintained on PDA agar slants at 4°C.

Phytochemical screening

The ethanol extract of AM leaves was subjected to prelimnary phytochemical screening using standard methods (16). The plant extract was screened to detect the presence of major phytoconstituents like alkaloids, saponins, flavanoids, glycosides, coumarone glycosides, tannins etc.

Determination of inhibition of fungal growth and aflatoxin production

Spores from the strains preserved on Czapek dox agar plate were obtained and inoculated into 5ml of the sterile 2% Tween 80 solution to prepare inoculum. 5ml of inoculum was transferred into synthetic production media (gm $lit^{-1}$of distilled water): Glucose, 20.00; yeast extract, 5.00;KH_2PO_4,1.52; K_2HPO_4, 1.00; KCl, 0.52; $MgSO_4.7H_2O$, 0.52; $CuNO_3.3H_2O$, trace; $ZnSO_4.7H_2O$, trace; $FeSO_4.7H_2O$, trace; tryptone, 20.00; peptone, 20.00; cas-aminoacid, 20.00; temperature, 25°C; agitation, 160 rpm; pH 7.5(after sterilization). The flask was then incubated on orbital rotary shaker (steelmate novatech, India) at 200 rpm at 25°C for 6 days and growth of fungi was monitored during incubation (4).

In order to check aflatoxin production in presence of AM extract, the test organism was grown at increasing concentration of AM powdered extract (0.5-2.0 mg/ml). The effect of extract was checked by monitering growth of mycelia and aflatoxin production. After incubation mycelia was separated by filtration and centrifugation of filtered broth was carried out to remove impurities.

Detection of aflatoxin by using TLC

Thin layer chromatography of broth was carried out with a objective to characterize the amount of aflatoxin produced, on precoated plates of Silica gel G 60 of dimension 10×10 cm. The filtered broth was tested for aflatoxin by using TLC and observing fluorescence at 360 nm by using the mobile phase Toluene: ethylacetate: chloroform: 90% formic acid (7:5:5:2). The sample (Fermented broth) was applied with the help of capillary about 2 cm from the bottom of the TLC plate. Chamber saturation was done by pouring the mobile phase (Toulene: Ethyl acetate: Chloroform: 90% Formic acid (7:5:5:2) into the glass chamber, covered it with glass lid and saturation was done for 30 minutes. The spotted plates, after air drying were kept in saturated chamber and solvent was allowed to run after 2/3 run. After that plates were removed, air dried and examined visually under U.V. light at 365 nm the solute front and solvent front were recorded to calculate R_F values and R_F values were compared with reference compound.

RESULT AND DISCUSSION

In the present investigation 4 fungal strains were isolated from the infected groundnut sample. Out of 4 strains a

Table 1. Screening of aflatoxin producing strains from infected ground nut and maize

Fungi	Growth	Aflatoxin
F1	+	-
F2	+	-
F3	+	–
F4	+	+

(+, growth; - no growth)

strain designated as F4, showed blue color fluroscence on TLC plate at 360 nm. To ensure toxigenic potential, production of aflatoxin was carried out by using shake flask method. The screened strain showed production of aflatoxin at shake flask level (Table 1). The plant extract showed the presence of phytoconstituents like alkaloids, saponins, flavanoids, glycosides, gums, and tannins with organic and inorganic constituents. The effect of different

Table 2. Effect of Aegle marmelos extract on growth and aflatoxin production on strain F4

Broth containing plant extract (mg/ml)	Empty petridish weight (gm)	Petridish with dried mycelia(gm)	Weight of mycelia (gm)
(Control)	48.140	56.64	8.5
0.5	48.140	55.86	7.72
1.0	48.140	55.02	6.884
1.5	48.140	53.92	5.784
2.0	48.140	50.68	2.54
Standard Griseofulvin (100µg/ml)	48.140	54.14	6.00

Table 3. TLC profile of strains F4 control and test flask at 2mg/ml of AM extract

Fungal strain	Flurosence under UV at 365 nm Control)	Flurosence under UV at 365 nm (Test)	RF values (control)
F1	Light Blue	Absent	0.525
	Light Blue	Absent	0.524
	Light Blue	Absent	0.524
	Light Blue	Absent	0.524

concentration of extract of AM leaves on growth of strain F4 is showed in (Table 2). The results show that inhibition of the fungal growth is related to the concentration of the AM extract. The growth of mycelia was maximum (8.5 gm) in a flask without AM extract whereas it was lowest (2.54 gm) in a flask containing 2mg/ml of AM extract and 6.00gm in flask containing standard antifungal griseofulvin at concentration of 100 µg /ml . TLC profile of the control and test broth of the strain F4 is summarized in (Table 3). No fluorescence in the test flask containing 2 mg/ml AM extract while it shows light blue color fluroscence was observed in the control flak (Table 3).The results show that antifungal activity of the extract is comparable to standard antifungal griseofulvin. The result shows that the ethanol extract of leaves of AM has potential to inhibit the fungal growth as well as it has profound antiaflatoxigenic activity. Our findings match with earlier studies of Kumar et. al, (17) and Mishra et.al (18). However systemic and concentration dependent study is required for the commercial exploitation of this plant as a natural remedy for controlling food born aflatoxicosis.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. D.R. Patil principal, R.C.Patel A.S.C. College, Shirpur for providing laboratory support.

REFERENCES

1. Koutsoumanis, K. Tassou, C.C Taoukis, P.S. Nychas, G.J.E. Modelling the effectiveness of a natural antimicrobial on Salmonella enteritidis as a function of concentration, temperature and pH, using conductance measurements. *J of Appl Microbiol* **84**: 981–987 (1998).
2. Lattaoui, N. Tantaoui, A. Elaraki. Individual and combined antibacterial activity of the main components of three thyme essential oils. *Rivista Italiana EPPOS*, **13**: 13–19 (1994).
3. Kabara J.J. Phenols and chelators. (In: Russell, N.J., Gould, G.W. (Eds.), *Food Preservatives*. Blackie, London), pp. 200–214 (1991).
4. Gourama H., Bullerman L.B. Antimycotic and antiaflatoxigenic effect of lactic acid bacteria a review. *J of Food Protect* **57**: 1275–1280 (1995)
5. Bathnagar D. Garcia S., Aspergillus.(In: Labbe, R.G., Garcia, S. (Eds.), *Guide to Foodborne Pathogens*. John Wiley and Sons, New York) pp.35–49 (2001).
6. Vardon P.J., Mycotoxins: risks in plant, animal and human systems, potential economic costs of mycotoxins in the United State. *Cast Task Force*: 136–142 (2003).
7. Leontopoulos D., Siafaka A., Markaki P., Black olives as substrate for *Aspergillus parasiticus* growth and aflatoxin B1 production. *Food Microbiology* **20**: 119–126 (2003).
8. Soliman K.M., Badeaa R.I., Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chem Toxicol* **40**: 1669–1675 (2002).
9. Rasooli I., Owlia P., Chemoprevention by thyme oils of Aspergillus parasiticus growth and aflatoxin production. *Phytochem* **66**: 2851–2856 (2005).
10. Ellis W.O., Smith J.P., Simpson B.K., Aflatoxins in food: occurrence, biosynthesis, and effects on organisms, detection, and methods of control. *Crit. Rev. Food Sci. Nutr.* **30**: 403–439 (1991).
11. Allocraft R., Aflatoxicosis in farm animals. In: Goldblat, L.A. eds., *Aflatoxin: Scientific Background, Control and Implications*. Academic Press, New York), vol **2** pp. 237–264, (1969).
12. Coulombe R.A. Jr., Biological actions of mycotoxins, *J. Dairy Sci.* **76**: 880–891 (1993)
13. Mishra H.N., Das C., A review on biological control and metabolism of aflatoxin. *Crit. Rev. Food Sci. Nutr.* **43**: 245–264 (2003).
14. Jayashree S., Subramanyam T., Antiaflatoxigenic activity of eugenol is due to inhibition of lipid peroxidation. *Lett. Appl. Microbiol*. **28**: 179–183 (1999)
15. S. K. Gond, Verma V. C., Kumar A Kumar V and Kharwar R. N. Study of endophytic fungal community from different parts of Aegle marmelos Correae (Rutaceae) from Varanasi (India). *World .J. Microbial Biotech* **23**: 1371–1375 (2007)
16. G.E. Trease. and W.C. Evans. In: Pharmacognosy 13th Edition. Bailliere Tindall Ltd London.
17. Singh P., Kumar A., Dubey N.K., Gupta R., Essential oil of *Aegele marmelos as* a safe plant based antimicrobial against post harvest microbial infestations and aflatoxin contamination of food commodities, *J. Food Sci*. **74**: 302–307 (2009)
18. Mishra B.B., Kishore N., Tiwari V.K., Singh D.D., Tripathi V., A novel antifungal anthroquinone from seeds of *Aegle marmelos Correae* (Rutaceae), *Fitoterapia* **15**: 746–749 (2009)