The study of spiritual remedies in orthodox rural churches and traditional medicinal practice in Gondar Zuria district, Northwestern Ethiopia

Muthuswamy Ragunathan[1]* Mequente Solomon[2]

[1] Department of Pharmacognosy, Cherraan's College of Pharmacy, 521- Siruvani Main Road, Coimbatore-39, Tamilnadu State, India

[2] Department of Pharmacology, Addis Ababa University, Medical Faculty, PO Box 9086, Addis Ababa, Ethiopia

ABSTRACT

During flowering season from September 2007 to December 2007, ethno medicinal information, as well as Spiritual remedies, documentation carried out among Orthodox Christian people of Gondar zuria district in northwestern Ethiopia. Based on the local utilization of plants and the aboriginal knowledge provided by the orthodox Christian people were documented. Field survey and personal discussion method has been used for collection of data. A list of 33 therapeutically, potential plants of 31 genera, 30 species belonging to 24 families identified by its taxonomic characters. The plants with their local names, ethno therapeutic claims including mode of preparation and method of applications to treat common illness and socio demographic profile of informants and spiritual remedies given by the Orthodox Christian priest in this district are studied.

Keywords: Medicinal Plants; Spiritual remedies; Orthodox Christian; Gondar zuria district; Ethiopia

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** Prof. Ragunathan Muthuswamy; mobile; 00919865656279
E-mail: ragunathranilmonica@gmail.com; ragunathan@fastmail.fm

INTRODUCTION

The World Health Organization (WHO) defines traditional medicine as health practices, approaches, knowledge, and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses and maintain well-being (1). In prehistoric culture, the mixture of herbal potion together with religious ritual and magic like formulas were part of all healing practices. Ethiopian society is not an exception to these traditional healing practices. Peoples here follow customs of Christian, Muslim, Jewish and other primitive religious rituals together. Today in Ethiopia up to 80% of the population uses traditional healing practices (2).

Most of the herbal remedies are interrelated to Ethiopian Orthodox Christianity religious practices. Priests of the church always taking the cross where ever they go and bless the people is usual here and they give massage over the disease-affected part with the cross. With the help of incantation, priest convert ordinary water to holy water, they spill it over the patients, and they enchantment spiritual words to drive away the evil spirit from the people those who suffered by evil attack. Apart from this, priests are giving the herbal remedies.

The fame of traditional religious-magical healings and herbal healings in villages is due to the lack of modern health care facilities. The statistical data shows the average physician to population ratio is 1:51042 and orthodox priests to people ratio is 1: 100(3).So, there is no surprise that, the local populations seeks traditional medicine from orthodox priests and consider the Bible as more than the doctors. These traditional religious healings practices are common in all the villages and it is easily reachable for the people commonly, economically and geographically.

2. MATERIALS AND METHODS

Ethnography of study Area

The Gondar zuria district is one among 18 districts in Amhara region in northwestern part of Ethiopia. It is at the distance of 700 km from northwest to Addis Ababa, the capital city of Ethiopia. This district is astronomically located at 12° 40'N latitude 370° 45'E- longitude. It lies at an altitude of 1550 to 1800 m above the sea level. It receives an annual rainfall of 711.8 to 1822 mm and monthly temperature from 18°c to 22°c. There are seven major native tribal groups in this district accounting, Agew 0.04%, Amhara 91.23%, Kement 8.25%, Tigraway 0.15%, Weyito 0.15%, others 0.15%. The total population size in this area is 258,146 among these males are 130,723; females are 127,423.The total area of Gondar zuria district is 1,286sq.km, with a population density of 200.6 per sq.km. As per topography, the major areas surrounded by hills and gentle slopes and partially bounded by higher elevation. The dominant soils here are shallow combisol, silt clay loam, and are brown in color. The soil depth is in between 20 to 70 cm. This area bordered by districts like Belesa at East, Denbya at West, Lay Armacheho, Wegera at north and Lake Tana and south Gondar in south (4).

Study Design

Ethno medicinal survey carried out during the flowering season that facilitated perfect identification of plants, as well as maximum availability of crude drugs in the market for sale, during these months. By using a semi structured questionnaires as per the standard guidelines (6).The interview carried out in Amharic language, which is the regional language of this district. Field trip made in villages of this district to collect the ethno medicinal information as well as the spiritual remedies from priests (spiritualist), traditional healers, herb sellers, farmers, and patients. After had first interview with informants, we again went after a month and taken second interview with same informants who we met in first meeting to get strong consent over name and usage of plants. For ethical consideration in each village, the local community leaders informed about the aim and the purpose of this study and requested to cooperate. Each informant asked to get consent verbally to participate in this study.

Sampling and Data Collection

The cross sectional study was made in Gondar zuria district of north west Ethiopia which includes major village settlements like; Gabrael, Sandaba, Netrha, Tekara, Azezo, Gonderoch Marium, Deg Abo, Laye Teda, Brehala Mecha, Abune Simra, Mardo, Jeja, Farka and Besan. The informants selected based on their traditional medicine knowledge. The volunteer informants were in the age group of 35 to 75 years. From this group the interviewed males number=93 (86.91%) are dominant, compared to females n=14 (13.08%). Among traditional healers n=19 all are men, priests n=55 all are men, farmers n=10(men 8, women 2), patients n=9 (men 6, women 3), herb sellers in the market n=14(men 5, women 9). All the informants were Orthodox Christians belonging to the Amharic ethnic group. With reference to the education status of informants, illiterate are n=36(33.64%), church educationist n=48(44.85%), before 1970, instead of going to school, students were studied, bible at church. Learn Geez'e language along with the rapeutic usage of plants, medico magical remedies, including fortune telling, techniques used to drive out evil spirit, amulet preparation, baptism, diagnose of diseases by using mirror etc. Primary school educationist n=15 (14.01%), Secondary school educationist n=8 (7.47%). The majority of informants gained healing knowledge, from their ancestors are n=47 (43.92%). From orthodox Christian churches are n=51 (47.66%). From their friends are n=2 (1.86%) and from the traditional healers are n=7 (6.54%). With reference to years of healing experience of traditional healers; 15 years and above are (68%), 12 years are (26.2%) and less than 10 years are (5.8%).

Apart from the socio demographic profile, informants interviewed for the standard questionnaires as to which plants and what parts of the plant, the local population uses and for which purpose; subsequently the plants of importance to the local people were documented. This includes local name of the plant, mode of preparation, route of administration and dose. At the end of the interview, plant specimen collected and identified by its taxonomic characters, and prepared herbarium deposited in Department of Pharmacognosy, School of Pharmacy, University of Gondar.

Data analysis

EPI info version 3.2 statistical package used for entry and calculating qualitative and quantitative data. Any logical and consistency error identified after revision of the original completed questionnaires. Facilities in MS excel spread sheet were utilized to make simple calculation, determining proportions and Informant consensus factor (ICF) values were determined to evaluate the consensus among traditional healers for potential anticancer plants.

RESULTS

This survey has documented about 33 plants of 31 genera, 30 species belonging to 24 families found to have utilized

in the traditional health care delivery system of the people in Gondar zuria district [Table 1]. Totally 37 different diseases including; all types of cancer, male impotence, jaundice, tonsillitis are treated by 42 formulations made by traditional medical healers from 33 plants. Among these leaves were in highest numbers n=24 (53.33%) followed by roots were n = 8 (17.78%), and seeds were n=6 (13.33%) barks were n=5 (11.11%) and whole plants were n=2 (4.45%).The main route of drug administration is oral n=29 (67.44%), followed by external application n=13 (30.23%) and inhalation n=1 (2.33%). For oral administration, a cupful of extract given and it was estimated 150ml. The most of the plant preparations were from single plant and the plant products were consumed in the form of decoction 21.42 % (9), juice 28.57 % (12), paste 38.09 % (16), infusion 7.14 % (3), oil 2.38 % (7), suspension 2.38 % (1), are given orally. Macerated material paste mixed with butter or honey, applied externally. Among the Plant families, each five species belonging to Euphorbiaceae and Fabaceae respectively and two species recorded in the family of Celastraceae.

This study was found that four plants namely, *Acalyph acrogyna* Pax, *Carissa spinarum* L., *Maytenus ovatus* (Wight&Arn.) Loes., and *Macaranga capensis* (Bail.) Benth. Ex.Sim is using to treat all type of cancer in this district. A group of researchers in National Cancer Institute (NCI) USA isolated Maytansine from *Maytenus ovatus* (Wight &Arn) Loes, Celastraceae (6). In 1977, the above-cited institute carried out clinical trial with Maytansine at the same time a group of Anamycin antibiotics that are same structure to Maytansine were also isolated from a fermented broth of Nocardia (7). The ICF calculation of anticancer plants as follows, *Carissa spinarum* L., (ICF = 0.14) frequency 21 *Maytenus Ovatus* (Wight & Arn.,) Loes, (ICF = 0.11) 26 frequency *Acalyph acrogyna* Pax. (ICF = 0.16) frequency 18.

DISCUSSION

In ancient period, Egyptian Abushakir who wrote "Bahre-Hasab" (Ocean of mathematics) which has contains more than 2000 pages, written in Arabic language latter it converted into Gee'ze language, which describes many

Table 1. list of plants using by people of Gondar zuria district, northwestern Ethiopia

Plant name, Family & Local name in Amharic :A	Part used	Application & route of administration	Mode of preparation& Dosage
Acalypha acrogyna Pax.,Euphorbiaceae Juss A: Gullo	Leaf	All types of Cancer / oral	Fist of leaf is grinded with stone mortar; pestle, the resultant paste is mixed with honey, and given orally in morning. The leaf is heated and applied externally over the tumor-affected area until cure
Adansonia digitata L., Bombacaceae A: Bamba	Bark	Cholera, bloating of stomach /oral	A teaspoon of bark powder boiled with water to make decoction and a cup of this given orally thrice a day before food for a week.
Asparagus africanus var. *abyssinicus* Fiori.,Liliaceae A: setkest	Bark	Syphilis /oral	Pieces of bark immersed in local beer, tela for whole night and in following morning, the content is filtered and a cup this infusion is given orally for 15 days.
Brucea anti dysentrica J. F, Mill., Simaroubaceae A: Abalo	Leaf	Leprosy, scabies and skin diseases /external	Handful of fresh leaf grounded to make a paste and to it add small quantity of honey or butter and it is applied externally until cure.
	Leaf	Syphilis, chronic diarrhea./oral	A half cup of fresh leaf juice is given orally for one week
Calpurnia aurea Baker Fabaceae A: Digita	Leaf	Ascariasis, gastric ulcer /oral	Seven to ten leaves are boiled in water, filtered and a cup of this decoction is given orally in empty stomach, in the morning for a week.
Carissa spinarum L., Apocynaceae A: Agam	Leaf	Snake bite/external	A handful of leaf is grinded to make in to paste, which is applied over snake bitten area
	Twigs & leaf	Throat cancer /oral	Young twig and fresh leaf is collected, pounded to make paste, and mixed with honey, given orally until cure

(continued)

Plant name, Family & Local name in Amharic :A	Part used	Application & route of administration	Mode of preparation& Dosage
Caesalpinia decapetala [Roth.,] Alston., Fabaceae A: chinklate zer	Seed	To prevent false perception/ external	Seeds are used to make necklace, it is be worn prevent false perception and the plants grown as hedge around the house.
Croton macrostachyus Hochst. ex. Delile., Euphorbiaceae A: Bsana, Bisana	Bark	Gonorrhea, Abdominal colic./oral	A piece of fresh bark is soaked in 150 ml of water overnight and in the following morning a cupful of this infusion is given orally for a week.
Dodonaea angustifolia L.f. Sapindaceae Juss, A: kitkita	Leaf	Jaundice, malaria and taeniasis./oral	A fist of leaf is grounded to get half a cup of juice, which is given orally in morning and evening until cure.
	Seed	Gastric pain, bowel colic/oral	A small quantity of seed powder mixed with honey to make paste, which is given orally in the |morning, and carrying little seed used to prevent evil eye.
Erythrina abyssinica Lam ex DC., Fabaceae A: Korch	Leaf	Bone fracture , skin wound /external	A handful of fresh leaf grounded to make a paste and it is applied and tied along with bamboos splints and bandage
	Root & bark	Indigestion /oral	Both root and bark pieces are immersed in local beer tela for whole night and in the morning the content is filtered and a cup of this infusion is given orally in empty stomach.
Guizotia abyssinica (L.F.) Cass.,Compositae A: Nug	Seed	Dry cough/oral	A cup of seed powder decoction is given orally in the morning and evening for a week.
	Seed oil	Rabies /oral	A cup of oil is given in morning in empty stomach until cure.
Lagenaria abyssinica (Hook.F.) C. Jeffrey Cucurbitaceae. A: Emboy yekil	Whole plant	Bronchitis, pneumonia /inhalation	Inhale the smoke of the whole plant in morning and evening for a week. Whole plant used to ferment the preparation of local spirit Arake.
	Leaf	Fungal infection / external	Leaf paste is applied over the fungal infected area.
Lupinus albus L., Fabaceae A: Gibto	Seed Fruit	Hypertension /oral	Small quantity of seed and fruit is grounded with water, filtered. The resultant juice is given orally in morning for one month.
Macaranga capensis (Bail.,) Benth.Ex. Sim Euphorbiaceae A:Yehail shirr	Root	Male impotence/oral	A piece of fresh root is grounded in morning and put in the local beer tela and keep the content aside for seven hours, and in the evening a cup of this infusion is given orally
Maytenus Ovatus (Wight & Arn.,) Loes., Celastraceae A: Atat	Leaf	All types of cancer/oral	A handful of leaves are minced to make paste and a small quantity of this paste mixed with honey and given orally for morning and evening till cure
Maytenus undata (Thumb.,) Blake lock. Celastraceae A: Checho	Leaf	Eye infection /external	Three drops of fresh leaf juice instill into the eye
Ocimum lamiifolium Hochst Ex. Benth. Labiatae A: Damakesse	Leaf	Cold, cough ,fever and Antidote for Poison / oral	A handful of fresh leaf is pounded and a cup of this juice is given orally in the morning and night till cure
Osyris quadripartite Decn., Santalaceae A: keret	Leaf	Jaundice/oral	A handful of fresh leaf is grinded and cup of this juice given orally for 15 days

(continued)

Plant name, Family & Local name in Amharic :A	Part used	Application & route of administration	Mode of preparation& Dosage
Piper capense L.f., Piperaceae A: Timiz, Tmz	Seed	Cold ,cough & Stomach ache /ora	A teaspoon of seed powder mixed with a cup of milk and it is given orally for a week in morning. The seed powder also used to make food sauce.
Piliostigma thonningii (Schumach. & Thonn.) Milne-Redh. Fabaceae A: Wanza	Bark	Wound heal after male, female circumcision/external	A piece of bark is grounded with water to make paste. It is applied over the wound after circumcision.
Prunus Africana(Hook.f,) Kalkman., Rosaceae A: Tikur Inchet	Leaf	Tonsillitis/oral	A fist of fresh leaf is pounded with water and a half cup of this juice is given orally in morning for one week
Rubia cordifolia L, Rubiaceae A: Enchibir	Root & leaf	Cold ,cough fever /oral	A teaspoon each of both root and the leaf powder is put in the boiling water, filtered and a cup of this decoction given orally in morning and evening for seven days.
Rumex nepalensis Spreng., Polygonaceae A:Ye wusha lut	Root	Ascariasis abdominal bleeding and gastric ulcer /ora	A teaspoon of root powder is boiled in 150 ml of water to make a decoction. A cup of this decoction is given orally in the morning for fifteen days in empty stomach.
	Leaf	External hemorrhage and healing the wound/external	A fist of leaf is grounded to make a paste and applied externally.
Rhamnus prinoides L'Her. Rhamnaceae A: Gesho	Leaf	Leaf part used to prepare local beer	Leaf is the one of the material for fermentation, which is used to prepare the local beer tela.
	Seed	Tinea- ringworm/oral	A cup of seed decoction is given orally in morning for 1 week.
Rosmarinus officinalis L.,Lamiaceae Qora.	Whole plant	Neuritis and paralysis/ oral	Fresh leaf juice is given orally in morning for forty days.
Snowdenia Polystachya [Fresen.,]Plig.,Poaceae A: Muja	Leaf	Tinea -ringworm / external	A part of the young plant is grounded with water and a small quantity of the resultant paste is applied externally.
Salvia nilatica Juss.Ex Jacq. Lamiaceae A:keskeso	Leaf	Wart/external	Fresh leaf is grounded with water to make a paste, which is applied externally over the wart-affected area.
Signa abyssinica, Hochst. Ex.Rich., Caryophyllaceae A: D" nbilal	Leaf	Skin wound /external	Fresh leaf paste is applied over the wound.
	Leaf	Stomach ache/oral	A fist fresh leaf is grounded with water and a cup of this juice given orally in morning and evening until the pain is relieved.
Syzygium guineense [Wild.] Dc.,Myrtaceae A: Anqa, Doqma	Root & leaf	To treat syphilis and stomach ache./oral	A decoction is made from each one teaspoon of root and leaf powder and a cup of this decoction is given orally three times a day for seven days.
Securidaca longepedunculata Fresen., Polygalaceae A: Etse menahe	Leaf	To get rid of tooth pain/oral.	Chew and sip the leaf juice in morning and evening.
		To treat snake bite/oral	A cup of fresh leaf juice is given orally and leaf paste applied over the bitten area.

(continued)

Plant name, Family & Local name in Amharic :A	Part used	Application & route of administration	Mode of preparation& Dosage
Tragia pungens [Forssk.,] Mull, Arg., Euphorbiaceae A: Aleblabit	Root & Leaf	Chronic cough(T.B) impotence/oral	A teaspoon of leaf and root powder is boiled in water to make decoction. A cup of this is given orally three times a day for forty days in the morning and evening.
	Leaf	snake bite external	Leaf paste is applied externally over the snake bitten area.
Tragia cinera [Pax] M.G.Gilbert & Radcl.- Smith.,Kew Bull., Euphorbiaceae A: Tinkushta	Root	To relieve pain in scorpion sting/external	Large pieces of fresh root are grounded in to paste with the help of stone mortar and pestle. It is applied over the affected area.
Vernonia adoensis Sch .Bip. Ex. Walp., Asteraceae Yetota shug	Leaf	For healing the wound/external	Fist of fresh leaf are minced and resultant paste is applied externally.
	Root	Snake bite/oral	Chew up the root, sip the juice, and fumigate the root to inhale.

medico magical remedies and astronomical calculations. In a similar manner, another book compiled with a title "Metshafe-Tibeb" (Book of wisdom) which describes usage of many medicinal plants along with spiritual remedies. The copies of these books are avail in many churches in northwestern Ethiopia. This study also found that, based on medical description given in the above cited books many priest practicing traditional medicine. Most of the Orthodox Churches, practicing Jewish traditional customs like male circumcision.

People believe that health is a 'gift of God' and 'evil forces can cause diseases this make community rely on spiritual remedies. Due to this believe, the influence of orthodox Christianity has many religious-magical healing practices, which are very popular among every Ethiopian. This study confirmed that, medico magical remedies of *Dodonaea angustifolia* L.F., the seeds of this plant are using to prevent evil eye (Buda).With help of seeds *Caesalpinia decapetala* (Roth.) Alston, women make and wear necklace to prevent false perception. The primary form of well-accepted traditional religious-magical healing for every Orthodox Christian is the Holy water ('tsebel'). Blessed holy water used to drink for healing diseases. Other popular healing methods in church are baptism, fasting, and penance. In addition, priest giving some sort of counsel and guidance. Apart from this the soul-father, (yenafs abbat) is a kind of family spiritual-doctor makes frequent visits to the home and performs healing services as required.

CONCLUSION

This study has documented spiritual remedies along with ethno medicinal plants used by the community for various illnesses. Most of the medicinal plants are collected from wild and the leaves and roots are the frequently used plant parts, which might aggravate loss of bio diversity in the end. Therefore, promoting cultivation of medicinal plants by the community may reduce destruction of wild plants. Identity and study of endangered species of medicinal plants is necessary. This survey could contribute partial remedies in the preservation of Ethiopian cultural heritage.

ACKNOWLEDGEMENTS

The author is thankful to Dr. Azmal Hussein, Taxonomist, Biology department, Gondar University for his valuable support in plant identification.

REFERENCES

1. World Health Organization (2001).
2. Central statistical authority of Ethiopia (2000).
3. Ministry of Health, Ethiopia (2004).
4. *Statistical abstract*, July (Central statistical authority of Ethiopia, 2005) 250
5. Martin G. J, *Ethno botany A Methods and Manual*, (Chapman and Hall, London, 1995) 80.
6. Kupchan S.M., Komoda Y., Court W. A., Thomas G. J., Smith R. M. Karim A. Gilmore C. J., Haltiwagner R. C., and Bryan R. F. Maytansine a novel antileukemic ansa macrolide from Maytenus ovatus. *J. Am. Chem .Soc.* **94**: 1354–1356. (1972).
7. Higashade E., Asai M., Oatsu K., Tnida S., Kozai Y., Hasegawa T., Kishi T., Sugino Y. and Yoneda M., Ansamine, a group of novel maytansinoid antibiotics with antitumor properties from nocardia, *Nature*. **270**: 721–722 (1977).

In vitro Antioxidant Activity of *Moringa pterigosperma* (Gaertn) leaves.

Lobo Vijaya*, Phatak Anita, Varghese Jossy, Chandra Naresh.

Department of Botany, Birla College, Kalyan 421304, Maharastra. India.

ABSTRACT

The human body produces reactive oxygen species (ROS) as a result of normal metabolic process. These ROS are capable of oxidizing biomolecules that can damage DNA, cells and contribute to chronic disease. In treatment of these diseases, antioxidant therapy is gained an utmost importance. The important role of dietary antioxidants maintaining the integrity of the living organisms is gaining ever increasing recognition. The oxidative stress induced due to ROS can be attenuated or perhaps reversed by diets containing vegetables that have an ability to scavenge reactive oxygen species. *Moringa pterigosperma* (Gaertn) leaves has been used in Indian cooking and herbal remedies. Its possible mechanism of action was examined in terms of antioxidant availability. The study was designed to evaluate and compare the antioxidant activity, total phenolics, flavonoids content of aqueous (AEMP) and ethanolic extract (EEMP) extracts of leaves. The antioxidant activity was assessed by DPPH (1, 1–diphenyl–1,2–picryl hydrazyl), nitric oxide and superoxide radical scavenging assay, FRAP (Ferric Reducing Antioxidant Power), reducing power and TAC (Total antioxidant capacity). AEMP has shown higher antioxidant activity as compared to EEMP in DPPH radical scavenging assay with IC_{50} values 3649.63±1.81 in AEMP and 3048.78±1.23 in EEMP respectively. Like antioxidant activity the reducing power and FRAP values of AEMP are better as compared to EEMP. The amounts of total phenolic and flavonoid content were also determined. In conclusion, the studies reveal that *Moringa pterigosperma* Gaertn. can scavenge radicals and reduce iron complex may explain the possible mechanism by which it exhibits beneficial effects.

Keywords: *Moringa pterigosperma* , Antioxidant, DPPH, FRAP, *In vitro*.

Editor: Mueen Ahmed KK, Phcog.Net

***Correspondent author:** E-mail address: vijayalobo@rediffmail.com

INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench and/or scavenge them and protect the body against their deleterious effects (1). The ROS inducing superoxide anionic radical ($O_2^{\cdot-}$), hydrogen peroxide (O_2^{-2}) and hydroxyl radicals (·OH) are implemented in oxidative damage to various cellular macromolecules. Increasing number of evidence suggested that oxidative stress induced biochemical changes are crucial etiological factors in several chronic human disease such as diabetes mellitus, cancer, atherosclerosis, arthritis, inflammation and neurodegenerative disease (2). There have been many studies undertaken on how to delay or prevent the onset of these diseases. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Foods from plant origin usually contain natural antioxidants that can scavenge free radicals. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and / or chelating the catalytic metal ions. Several synthetic antioxidants, e.g. BHA and BHT are commercially available but they are suspected to cause or prompt negative health effects, and also show low solubility and moderate antioxidant activity. Natural antioxidants, especially phenolic and flavonoids are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption (3). *Moringa pterigosperma* Gaertn. (Moringaceae) is a small or medium sized tree, about 10m. high, found wild in sub Himalayan tract, from Chenab eastwards to Sarda, and

cultivated all over plains of India. All the parts of the tree are considered medicinal and used in treatment of ascites, rheumatism, venmous bites and as cardiac and circulatory stimulants (4). Leaves are anti inflammatory, anodyne, anthelmintic, ophthalmic and rich in vitamins A and C (5). Reports indicate that pharmacological activites of *Moringa pterigosperma* (Gaertn) leaves includes antitumour, radioprotective, antihypertensive, hypoglycemia, diuretic and hypocholestemia activities (6) and diabetes (7). *Moringa pterigosperma* Gaertn. has also been reported to possess antihepatotoxic, hypothermic and myocardial depressant activity (8). Therefore, the objective of the study was to investigate the phytochemical profile and *in vitro* antioxidant activity of *Moringa pterigosperma* (Gaertn) leaves.

MATERIALS AND METHODS

Chemicals

Trolox (6-hydroxy-2, 5, 7, S-tetramethylchromam-2-carboxylic acid) was purchased from Sigma Chemical Co. Ltd USA.. DPPH (1, 1 – diphenyl – 1, 2 – picryl hydrazyl), TPTZ (2, 4, 6,-tripyridy-s-triazine), potassium ferricyanide, trichloroacetic acid (TCA), $FeCl_3$, sodium nitroprusside, sulphanilamide, napthylethylenediamine dihydrochloride, TPTZ(2, 4, 6,-tripyridy-s-triazine), ascorbic acid, NBT (nitroblue tetrazolium), reduced NADH (nicotinamide adenine dinucleotide), PMS (phenazine methosulfate), sulphuric acid (H_2SO_4), ammonium molybdate, ascorbic acid/standard Vitamin C (Vit. C), quercetin and pyrocatechol was purchased from HiMedia, Mumbai. All other unlabelled chemicals and reagents were of analytical grade and used without further purification.

Plant material

The leaves of *Moringa pterigosperma* (Gaertn) were collected from Birla college campus, Kalyan, Thane district, Maharastra, India. The plant material was taxonomically identified by Blatter Herbarium St Xavier's College, Mumbai. A voucher specimen (No. 4891 of N.A Irani) has been preserved in a laboratory for further reference. The collected plant was dried under shade and powdered with a mechanical grinder and stored in an air tight container. The dried powder material of the leaves was soaked in distilled water and ethanol for 10hrs, to get an aqueous (AEMP) and ethanolic extract (EEMP) after filtration through Whatman paper No. 42.

Preliminary phytochemical screening

Qualitative phytochemical analysis of AEMP and EEMP was carried out as follows: Phenolics: 2ml of filtrate + 2ml $FeCl_3$, blue precipitate indicated presence of phenolics. Saponins (frothing test): 0.5 ml filtrate +5ml distilled water); frothing persistence indicated presence of saponins. Alkaloids: 2ml of filtrate +1%HCl+Dragendroff reagent, orange precipate indicate the presence of alkaloids. Flavonoids; 5ml dilute ammonia was added to a portion of filtrate +concentrated sulphuric acid; yellow colour indicates presence of flavonoids. Steroids (Liebermann-Burchard reaction: 2ml filtrate +2ml acetic anhydride +concentrated sulphuric acid; green color indicates the presence of steroids. Terpenoids: 4ml of filtrate +concentrated sulphuric acid 3ml was added to form a layer; reddish brown colouration interface indicates the presence of terpenoids. Cardiac glycosides (Keller-Kinliani test): 2ml filtrate + 1ml of glacial acetic acid + $FeCl_3$ +concentrated H_2SO_4; brown colour indicates the presence of cardiac glycosides (9).

Total phenolic content

The total phenolic content of different extracts was measured using colorimetric Folin –Ciocalteu method. The reaction mixture consisted 5ml of diluted sample to which 3 ml of distilled water and 0.5 ml Folin –Ciocalteu reagent was added. After 3minutes, add 2ml of 20% Na_2CO_3 solution and place the tubes in boiling water bath for one min, cooled and the absorbance was measured at 760 nm. Standard graph was prepared by using different concentration of pyrocatechol (10).

Total flavonoid content

The flavonoid content of different extracts was measured using a modified colorimetric method. 0.5ml of sample was mixed with 0.5 ml of 2% $AlCl_3$ and incubated for 10mins. and the absorbance was measured at 415 nm. The measurement was compared to a standard graph for quercetin (11).

Antioxidant Activity
Determination of reducing power (Fe^{3+} – Fe^{2+} transformation ability)

The reducing power of a compound serves as significant indicator of its potential antioxidant activity. Increased absorbance of the reaction mixture indicates increased reducing power.

Various conc. of the extracts in 1ml of water were mixed with phosphate buffer (2.5 ml, 0.2 M pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50M°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged al 3000g for 10 min. upper layer of

solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5ml, 0.1%). The absorbance was measured at 700nm (12).

Super oxide anion scavenging activity

1ml of NBT solution (144µM in 100mM phosphate buffer, pH 7.4), 1ml of reduced NADH (677µM in100mM phosphate buffer, pH 7.4) and 0.5 ml of sample extract was mixed and the reaction was started with adding 100µl of PMS solution (60µM PMS in100mM phosphate buffer, pH 7.4) . The reaction mixture was incubated at 25°C for 5 min, and the absorbance 560 was measured against blank (13).

$$\% \text{ scavenging} = \frac{A_{con} - A_{test}}{A_{con}} X\ 100$$

Nitric oxide radical scavenging activity

Sodium nitroprusside (5mM, 1ml) in phosphate buffer saline (PBS) (0.1 M, 7.4 pH) was mixed with 3 ml of different conc. of the extract and incubated at 25°C for 150 min. 0.5 ml of the samples was mixed with 0.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). Measure the absorbance at 546 nm (14).

$$\% \text{ scavenging} = \frac{A_{con} - A_{test}}{A_{con}} X\ 100$$

DPPH radical scavenging activity

The assay is based on the measurement of the scavenging ability of antioxidant towards the stable radical DPPH. DPPH radical react with suitable reagent, the electrons become paired off and the solution looses color stoichiometrically depending on number of electrons taken up.A volume of 2ml of sample was added to 2ml of phosphate buffer (0.02M, pH 6) and 2ml of 0.2mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min. at R.T. and the absorbance was measured at 517 nm (15).

The capability to scavenge the DPPH radical was calculated using following equation:

$$\% \text{ scavenging} = \frac{A_{con} - A_{test}}{A_{con}} X\ 100$$

FRAP assay

The stock solutions of 10mM TPTZ in 40 mM HCl, 20mM $FeCl_3.6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml of acetate buffer . It was prepared freshly and warmed at 37°C. 900µl of FRAP reagent was mixed with 90 µl of distilled water and 30µl of sample solution. The reaction mixture was then incubated at 37°C for 30 min and absorbance was recorded at 595 nm. The concentration of $FeSO_4$ was in turn plotted against concentrations of the standard antioxidants (L-ascorbic acid and Trolox) (16).

Total antioxidant capacity

0.1ml of extract was combined in eppendorf tube with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in thermal block at 95°C for 90 minutes. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695 nm against blank (17).

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance i.e. one way ANOVA and student't' test using GraphPad QuickCalcs.. The two-tailed $P < 0.05$ the difference is considered to be statistically significant and if $P < 0.0001$ the difference is considered to be extremely statistically significant The IC_{50} values were calculated from linear regression analysis.

RESULT AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening of AEMP and EEMP revealed the presence of various bioactive components like phenolics, saponins, flavonoids, alkaloids, terpenoids, cardiac glycosides. The oil from the seeds of *Moringa oleifera* is applied externally for skin diseases and this is due to the presence of terpenoids, as terpenoids strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply (18).

Total phenolic and flavonoid content

Total phenolic compounds are reported as pyrocatechol equivalents. The total phenolic contents of phenolics content of AEMP and EEMP were 4.44 ± 0.04 and 3.84 ± 0.03 mg pyrocatechol equivalent/g of sample, respectively. The total flavonoid contents of AEMP and EEMP were 6.30 ± 0.03 and 4.31 ± 0.02 mg quercetin equivalent/g of sample. AEMP had higher total phenol

and flavonoids contents than EEMP. It has been reported that green leafy vegetables, soft fruits and medicinal plants exhibited higher levels of flavonoids (19). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (20).

Antioxidant activity

Antioxidant activity of the extracts of varying concentrations ranging form 10- 10000 µg/ml was evaluated by various in vitro models. It was observed that the test compounds scavenged free radicals in concentration dependent manner in all the models.

The result of FRAP and TAC assay were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) and Ascorbic acid Equivalent Antioxidant Capacity as described. TEAC is the concentration of Trolox (µmol/L) required to give the same antioxidant capacity as 1% (w/v) test substance. AEAC is the same for Ascorbic acid (21).

Determination of reducing power (Fe^{3+} - Fe^{2+} transformation ability)

In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700nm (22). Increasing absorbance at 700 nm indicates an increase in reductive ability. Table 1 shows that the reducing powers of all the extracts and standards also increased with the increase of their concentrations. There was a extremely significant difference ($p< 0.001$) among the AEMP and EEMP in reducing power. The activity of AEMP and EEMP was not comparable with Vit. C and trolox ($p< 0.05$) there were significant differences between them.

Table 1 Reducing power of AEMP, EEMP, Vit C and Trolox

conc. (µg/ml)	Absorbance at 700nm			
	AEMP	EEMP	Vit C	Trolox
5	0.07±0.01	0.04±0.01	0.20±0.01	0.11±0.01
10	0.11±0.01	0.08±0.01	0.23±0.02	0.13± 0.01
50	0.15±0.02	0.11±0.01	0.37±0.01	0.16± 0.02
100	0.19±0.01	0.15±0.02	0.48±0.02	0.2±0.001
500	0.21±0.01	0.17±0.01	0.88±0.03	0.58±0.02

Values are means ± S.D. (n=4)

Super oxide anion scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes (23) of body as well as via nonenzymatic reaction such as autoxidation by catecholamines (24). In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm (25). Table 2 shows the superoxide scavenging effect of AEMP in comparison to EEMP on the PMS/NADH-NBT system. The increase of percentage scavenging activity thus indicates the consumption of superoxide anion in the reaction mixture by the plant extracts. Maximum percentage scavenging activity showed by EEMP is 16.17 ± 1.10 at 1000 µg/ml. Whereas AEMP showed 78.57± 3.10 at 10000 µg/ml. AEMP had strong superoxide radical scavenging activity as compared to EEMP which does not show IC_{50} value whereas AEMP gives IC_{50} value at 3875.96± 1.94 µg/ml. There were no significant differences ($p > 0.05$) between AEMP and EEMP. The IC_{50} value of Vit C is 66.31± 3.14, whereas Trolox shows at 2857.14± 1.01 µg/

Table 2 % Radical scavenging activity of AEMP and EEMP in different *In vitro* Assays at different concentrations

conc. (µg/ml)	% scavenging activity					
	Superoxide anion radical		Nitric Oxide radical		DPPH radical	
	AEMP	EEMP	AEMP	EEMP	AEMP	EEMP
10	0.90±0.01	7.17±0.16	3.05±0.71	7.07 ±0.78	10.15±2.14	2.10±0.70
50	7.12±0.71	9.19±1.28	10.11±1.08	18.17±0.91	15.18±1.17	3.18±1.28
100	12.01±1.78	7.17±1.00	28.27±0.91	50.01±2.78	17.18±2.28	20.17±2.17
500	28.17±1.01	13.18±2.16	31.31±2.01	75.04±0.46	20.15±2.18	28.98±0.18
1000	38.09 ±1.91	16.17±2.10	46.15±1.47	75.04±3.01	28.98±2.74	40.18±1.7
5000	57.14±0.21	14.17±1.10	81.07±0.71	75.04±2.01	77.78±1.11	60.61±1.10
10000	78.57±1.10	13.17±2.10	81.11±1.78	75.04±1.01	77.78±1.11	60.61±1.11

Values are means ± S.D. (n=4)

ml. Lower the IC_{50} value of better is the scavenging ability of the sample.

Nitric oxide radical scavenging activity

The extract effectively reduced the generation of nitric oxide from sodium nitroprusside (Table 3). In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant drugs. 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 (26). Both AEMP and EEMP decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro which may be due to the presence of antioxidant principles in the extract.The percentage scavenging activity increased with increasing concentration of the extract. Lower the IC_{50} value of better is the scavenging abitity of the sample. There were no significant differences ($p > 0.05$) between AEMP and EEMP. The IC_{50} value of AEMP and EEMP was found to be 2793.29±1.63 µg/ml and 100.51±1.21µg/ml, However, Std Vit. C activity of was very more pronounced than that of our extracts (162±1.32µg/ml.)

DPPH radical scavenging activity

DPPH assay is one of the most widely used methods for screening of antioxidant activity of plant extracts (27). DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenyl picryl hydrazine, with the addition of all fractions in a concentration-dependent manner. All the concentraction of AEMP and EEMP demonstrated H-donor activity. Lower the IC_{50} value of better is the scavenging ability of the sample. The IC_{50} values of AEMP and EEMP were 3048.78±1.23µg/ml and 3649.63±1.18µg/ml respectively. These activities are less than that of the Std Vit C and Trolox i.e. 10.40±1.43µg/ml and 17.42±1.21µg/ml respectively (Table 2). There were no significant difference ($p > 0.05$) between AEMP and EEMP.

FRAP assay

In FRAP assay the ability of plant extract to reduce ferric ions was determined. FRAP assay measures the changes in absorbance at 593 nm owing to the formation of blue colored Fe^{+2}- tripyridyltriiazine compound from the colourless oxidized Fe^{+3} form by the action of electron donating antioxidants (28). The FRAP values of AEMP is higher as compared to EEMP (Table 3). Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present it can be reported that AEMP and EEMP may act as free radical scavenger, capable of transforming reactive free radical species into stable nonradical products.

Table 3 FRAP and TAC value of AEMP and EEMP

1% (w/v) extracts used	FRAP Values (µmol/L)	TAC Values (µmol/L)
AEMP (AEAC)	0.57±0.02	0.59±0.01
EEMP (TEAC)	0.30±0.03	2.86±0.01

Values are mean ± S.D (n=4)

Total antioxidant capacity

Total Antioxidant capacity of AEMP and EEMP is shown in Table 3. The phosphomolybdenum method was based on reduction of MO (VI) to MO (V) by the antioxidant compound and the formation of green phosphate/ MO (V) complex at acidic pH (29). In this assay EEMP was found to have higher activity, AEMP showed lower activity. The extracts demonstrated electron donating capacity and thus they may act as radical chain terminators, transformating reactive free radical species into stable non reactive products (30).

CONCLUSION

The results of present study demonstrate that the radical scavenging of AEMP, indicate that the use of *Moringa pterygosperma* (*Gaertn*) for the treatment of diabetes, hypoglycaemia and hypertension treatment seems quite useful and reasonable. The percentage scavenging activity (with reference to IC_{50} value) in DPPH and super oxide anion radical scavenging assays shows that AEMP has better percentage scavenging activity as compared to EEMP. AEMP thus also give effective reducing power and FRAP values, But the TAC values AEMP are lower as compared to EEMP. The overall better antioxidant and free radical scavenging activities of AEMP might be due to the presence higher amounts of phenolic and flavonoid compounds in aqueous extract. Further studies are in progress in our laboratory to evaluate the in vivo antioxidant potential of this extract in various animal models and phytochemical studies are required to establish the types of compounds responsible for the bioactivity of this medicinal plant.

REFERENCES

1. Shirwaikar A., Shirwaukar A., Rajendran K. and Punitha I.S.R. *In vitro* antioxidant studies in the benzyl tretra isoquinoline alkaloid berberine. *Bio. Pharma Bull.* **29** (9): 1906–1910 (2006).
2. Soni H., Pandey H., Phatak A.K., Nayak G., Singhai A.K., Parihar A., Singh V. and Rathur A.S. Evaluation of antioxidant potential of hydro

alcoholic extract of leaves of *Coleus aromaticus*. *Adv. Pharmacol Toxicol*., **10** (1): 75–82 (2009).

3. Jain S., Gupta A., Malviya N. and Suhur H. Comparative antioxidant potential screening of polyherbal formulations. *Adv Pharmocol. Toxicol*. **10** (1): 101–110 (2009)
4. Thacker M. S. *Moringa*, The Wealth of India, Council of Industrial and Scientific Research, New Delhi. (1962) 426–429.
5. Sheth A. K. *Moringa oleifera*. *The Herbs of Ayurveda*. Vol **III** (2005) 275–776.
6. Hus R., Midcap S., Arbainsyah and DeWitte L. *Moringa oleifera; Medicinal and socio-economic uses*. *International course on Economic botany*. National Herbarium Leiden, The Netherlands, (2006) 13.
7. Jaiswal D., Rai P.K., Kumar A., Mehta S. and Watal G. Effect of *Moringa oleifera* Lam. Leaves extract therapy on hyperglycemic rats. *Journal of Ethano pharmacology*. **123** (3): 392–396 (2009).
8. Ross I. A., *Moringa pterygosperma (Gaertn) Medicinal plants of the world*. (Humana Press, Inc, New Jersey, 2003) 367–381.
9. Parekh J. and Chanda S. V. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol*. **31**: 53–58 (2009).
10. Sadashivam S. and Manickam A. *Phenolics*. *Biochemical Methods*. New age publishers, (New Delhi) 193–194 (2004).
11. Tilak-Jain J. *Studies of the antioxidant effects and other benefical properties of natural compounds from Indian medicinal plants*. Ph.D. thesis. Mumbai University, Maharastra, 2005.
12. 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).
13. Shivkumar H., Ansri S. A., Parkash T., Rao R. N., Swamy H.M.J. and Manjunath K.P. *Adv. Pharmacol. Toxicology*. **7** (1): 39–41 (2006).
14. Ilavarasan R., Mallika M. and Venkataraman S. Anti inflammatory and antioxidant activites of *Cassia fistula Linn* bark extract. *Afr. J. Trad*. **2** (1): 70–85 (2005).
15. Chen G., Zhao L., Zhao L., Cong T. and Bao S. *In vitro* study on antioxidant of peanut protein hydrolysate. *J. Sci. Food Agric*. **87**: 357–362 (2007).
16. Pulido R., Bravo L. and Calixto F.S. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food and chem*. **48**: 3396–3402 (2000).
17. Shirwaikar A., Prabhu K. and Punitha I. S. R. *In vitro* antioxidant studies of *Sphaernthus indicus* Linn. *Indian J. Expt. Bio*. **44**: 993–996 (2006).
18. Krishnaiah D., Devi T., Bono A. and Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plant. Res*. **3** (2): 067–072 (2009).
19. Sultan B. and Anwar F. Flavonols(Kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. *Food Chemistry* **108** (3): 879–884 (2009).
20. Van Acker S.A.B.E., van Den Berg D.J., Tromp M.N.J.L., Griffioen D.H., Van Bennekom W.P., Vander Vijgh W.J.F. Structural aspects of antioxidant activity of flavanoids. *Free Radical Biology and Medicine*. **20** (3): 331–342 (1996).
21. Gil M.A., Thomae- Barberan F. A., Hess-Piecrce B., Holcraft D. M. and Kader A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem*. **48**: 4581–4589 (2000).
22. Ebrahimzadeh M.A., Hosseinimehr S.J., Hamidinia A. and Jafari M. Antioxidant and free radical scavenging activity of Feijoa sallowiana fruits peel and leaves. *Pharmacologyonline* **1**: 7–14 (2008).
23. Sainani G.S., Manika J.S., Sainani R.G. Oxidative stress: a key factor in pathogenesis of chronic diseases. *Med Update*. **1**: 1 (1997).
24. Hemmani T., Parihar M.S. Reactive oxygen species and oxidative DNA damage. *Ind. J. Physiol. Pharmacol*. **42**: 440–44 (1998).
25. Khanam S., Shivprasad H.N., Kashama D. In vitro antioxidant screening models: a review, *Ind. J. Pharm. Educ*. **38**: 180–94 (2004).
26. Marcocci P.L., Sckaki A., Albert G.M., Antioxidant action of Ginkgo biloba extracts EGP761. *Methods in Enzymology*. **234**, 462–475 (1994).
27. Nanjo F., Goto K., Seto R., Suzuki M., Sakai M., Hara Y., Scavenging effects of tea catechins and their derivatives on 1,1- diphenyl-2-picryl hydrazyl radical. *Free Radical Biology and Medicine*. **21**: 895–902 (1996).
28. Gupta A. D., Pundeer V., Bande G., Dhar S., Ranganath I. R. and Kumari G. S. Evaluation of antioxidant activity of four folk antidiabetic medicinal plants of India. *Pharmacology online*, **1**: 200–208 (2009).
29. Saha M. R., Hasan S. M., Akter R., Hossain M. M., Alam M. S. and Mazumder M. E. H. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi Linn*. *Bang. J. Vel, Med*. **6** (2): 197–202 (2008).
30. Dorman H.J.D., Kosar M., Kahlos K., Holm Y., Hiltunen R. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varities and cultivars. *J Agric Food Chem*. **51**: 4563–4569 (2003).

Acaricidal Effect of *Plumbago zeylanica* L. Against *Amblyoma variegatum*

Kofi Annan[1]*, Rita Dickson[1], Abraham Mensah [1] and Theoplilus Christian Fleischer[2]

[1] Department of Pharmacognosy
[2] Department of Herbal Medicine; College of Health Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

ABSTRACT

Acaricidal effects of extracts and compounds derived from *Plumbago zeylanica* L. (Plumbaginaceae) roots against different stages of *Amblyoma variegatum* were assessed using impregnated filter paper bioassay and compared with that of the commercial gamma benzene hexachloride. The observed responses varied according to the stage in the life cycle. It was noted that 2.0% of the total methanolic extract of *P. zeylanica* roots applied resulted in 100% ($p<0.01$) mortality against the larvae while 4.0% resulted in 100% ($p<0.01$) mortality against the nymphs and adult ticks. Petroleum spirit and ethyl acetate fractions made from the crude methanol extract showed a dose-dependant action on the tick stages with the larva being the most susceptible. Isolation of the biologically active constituent from active fractions was done by using column and thin layer chromatography. The structure of the acaricidal component was analyzed by ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{13}C COSY-NMR and identified as plumbagin. The acaricidal activity of plumbagin was examined and found to be most pronounced on the larvae and least pronounced on the adult tick with LD_{50} of 3.7×10^{-2} and 1.37 mg/ml respectively ($p< 0.010$), while gamma benzene hexachloride (positive control) gave an LD_{50} values of 2.63×10^{-3} and 2.84×10^{-2} mg/ml against the larvae and adult ticks respectively. The results indicated that the naphthoquinone plumbagin possesses acaricidal properties thus giving scientific justification for the folkloric use of the plant in animal husbandry. β-sitosterol and stigmasterol isolated alongside plumbagin had no significant action on all stages of the tick.

Keywords: *Plumbago zeylanica*, *Amblyoma variegatum*, plumbagin, acaricidal.

Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** Tel. +233 274243641 Email: annankofi@yahoo.com

INTRODUCTION

Ticks and the diseases they transmit are widely distributed throughout the world, particularly in tropical and subtropical regions. It has been estimated that 80 percent of the world's cattle population is exposed to tick infestation (1). Although species of ticks and tick borne diseases (TBDs) differ among ecological regions, their impact on animal production is important wherever they occur. The tropical cattle tick, *Amblyoma variegatum* cause a large economic loss and serious problem to control since it can develop resistant strains against synthetic acaricides (2). Losses attributable to ticks are caused either directly, through tick worry, blood loss, damage to hides and udders and the injection of toxins, or indirectly through mortality or debility caused by the diseases associated with the ticks. Synthetic acaricides that are often used to control the ticks pose several challenges including cost, resistance and pollution of water bodies. Therefore new acaricidal sources especially from natural products that would address the problem of cost and be environmentally friendly would be most welcome.

There are many indigenous plants that have shown acaricidal activity. These include both higher and lower plants and their acaricidal actions have been attributed to different classes of chemical compounds (3, 4). In an attempt to investigate the local flora of Ghana for acaricidal activity, attention was drawn to *Plumbago zeylanica*, a decoction of which is used locally to control mites on domestic animals. This work therefore seeks to validate the ethnopharmacological use of the plant and to identify the active principle(s) responsible for the acaricidal effect(s).

METHODOLOGY

Collection and authentication of plant

Plant materials were collected in October, 2007, at the Physic garden of the Faculty of Pharmacy and Pharmaceutical

Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. It was authenticated at the Department of Pharmacognosy of the same institution, where a voucher specimen (003/10/07) has been deposited.

Extraction and isolation of active acaricidal principles

The dried powdered root (1.0 kg) was sohxlet extracted with methanol (2.0 litres) for 48 hours. The extract was concentrated using the rotary evaporator and further dried over nitrogen to give a yield of 1.6 %.

The crude methanolic extract was fractionated with petroleum spirit and ethyl acetate respectively by liquid-liquid partition method. The concentrated extract was partitioned between 3 × 100 ml petroleum spirit (60–80°C) and water (distilled) [1:1]. The petrol fraction was then separated and concentrated to give a solid friable mass, 2.4 g. To the remaining aqueous fraction 3 × 100 ml of ethyl acetate was added in three successions. This was shaken well to effect extraction and pooled together to give ethyl acetate fraction which was concentrated and dried to yield 5.2 g of solid friable mass. The rest of the aqueous fraction was freeze-dried and yielded 7.4 g. Chemical profiles established using thin layer chromatography revealed that both petroleum spirit and ethyl acetate fractions had similar profiles.

Four grammes of ethyl acetate fraction (active) was column chromatographed on silica gel (150 g) [Sigma, 70–230 mesh ASTM] and eluted with 5% v/v, 15% v/v and 50% v/v of ethyl acetate in petroleum spirit (60–80°C) and 100% ethyl acetate to give four eluates, A–D. Crystals formed after drying of eluate A (5% ethyl acetate in petroleum spirit) were recrystalized in petroleum spirit (40–60°C) to yield an orange-yellow needle-like crystals (3.3 g) and designated 1. Column chromatography of eluate B (15% ethyl acetate in petroleum spirit) resulted in a mixture of two compounds which were further separated by PTLC (12% ethyl acetate in petroleum ether) to yield compounds 2 (106 mg) and 3 (230 mg).

Bioactivity studies

Source of organisms

The unfed larvae, nymphs and adult ticks (*Amblyoma variegatum*) were obtained from the Animal Research Institute of the Centre for Scientific and Industrial Research (CSIR), Ghana.

Preliminary bioactivity of fractions

Preliminary bioactivity studies of crude methanolic extract, petroleum spirit, ethyl acetate and aqueous fractions were carried out using 48-hour unfed tick larvae (5). Serial dilutions of extract and fractions were prepared ranging from 2 – 0.002%. Whatman No. 1 filter papers were impregnated with various concentrations of the extracts. Fifty (50) active larvae were brought into contact with the impregnated filter paper and observed after 24 hours for mortality with the aid of a hand lens. A larva was recorded dead if it showed no sign of mobility or any form of activity.

Acaricidal activity of isolates

Known concentrations of the isolates were prepared using 1% polysorbate solution, from which serial dilutions were made and their effects on the different tick stages observed as previously. All experiments were carried out in triplicate. Gamma benzene hexachloride, a known acaricide was used as a reference standard while 1% polysorbate solution was used as a control in all experiments.

Statistical analysis

One way ANOVA was used for the comparison of the means. Results were expressed as LD_{50} (% error) compared to the control.

RESULTS AND DISCUSSIONS

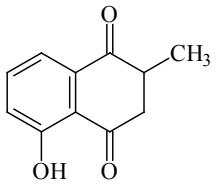
Compound [1] was crystallized out of petroleum spirit (40–60°C) as yellow needle-like crystals with melting point 78–79°C (uncorrected). The UV spectrum showed absorption at 210.2 nm, 265.4 nm and 417 nm which suggested a conjugated ring system (6). IR absorption peaks at Vmax; 1647.1, 1662.5 and 3566.1 cm^{-1} suggested the presence of a carbonyl and hydrogen-bonded hydroxyl groups respectively. The ^{1}H-NMR spectrum showed three aromatic proton multiplets at δ 7.50 (1H), δ 7.20 (1H) and δ 6.75 (1H) and an aromatic methyl doublet at δ 2.15 (3H). It showed a sharp proton singlet at δ 11.90 attributable to the hydrogen bonded hydroxyl group observed in the IR spectrum. The ^{13}C-NMR showed signals for four aromatic methines at δ 136.2, δ 124.2, δ 135.5, δ119.5; two keto-carbonyls at δ 184.8 and δ 190.3; a methyl group at δ 16.6; three quarternary olefinic carbons at δ 115.2, δ 132.2 and δ 149.7 and an oxygen-bearing quarternary carbon at δ 161.2. These data suggest a 1, 4-quinone structure fused with an aromatic ring.

In the HMBC spectrum, the proton at δ 7.25 (H-8) which directly correlated with the carbon signal at δc 119.5 in the HC-COBI spectrum, showed ^{2}J correlation with δc 132.2 (C-9) and δc 136.2 (C-7) and a ^{3}J correlation with δc 184.8 (C-1) and δc 136.2 (C-10) respectively.

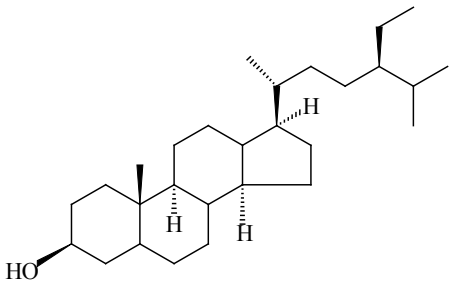
The aromatic proton at δ 7.55 (H-7) directly correlated with carbon signal at δc 136.2 in the HC-COBI spectrum whilst showing a ^2J correlation with δ 119.5 (C-8) and δc 124.2 (C-6) and a ^3J correlation with δc 132.2 (C-9) and δc 161.2 (C-5) in the HMBC spectrum. The proton signal at δ 7.20 (H-6) also directly correlated with carbon signal at δc 124.2 in the HC-COBI spectrum whilst showing a ^2J correlation with δc 161.2 (C-5) and δc 136.2 (C-7) and a ^3J correlation with δ 115.2 (C-10) and δc 119.5 (C-8). HMBC spectrum further revealed the hydrogen-bonded hydroxyl signal at δ 11.91 in a ^2J correlation with δc 161.3 (C-5) and a ^3J correlation with δc 124.2 (C-6) and δc 115.2 (C-10). The proton signal at δ 6.75 (H-3) was also found to be directly correlated with the carbon signal at δ 135.5 (C-3) and in ^2J and ^3J correlations with carbon signals at δ 190.3 (C-4) and δ 115.2 (C-10) respectively. The position of the aromatic methyl was unambiguously established by a ^2J correlation with the carbon signal at δ 149.9 (C-2) and a ^3J correlation with carbon signals at δ 184.8 (C-1) and δ 135.5 (C-3) in the HMBC spectrum. On the basis of the above spectral features [1] was characterized as plumbagin (5-hydroxy-2-methyl-1,4 naphthoquinone), a known compound previously isolated from the same plant species (7) and also identified from other plant species such as *Diospyros kaki* (8). The spectral data compared with those published.

On crystallization in chloroform, compound [2] was obtained as white needle crystals with melting point 138-139°C (uncorrected). IR (KBr) νmax: 3440, 2900-2850, 1430, 1370, 1615, 1051, 960 cm^{-1}. LR-EI-MS, *m/z* (rel. int., %) 414 (90), 396 (40), 351 (11), 303 (22), 255(33), 213 (22), 159 (20), 1449 (22), 107 (22), 55 (18). The EI-MS of [2] exhibited a maximum peak at *m/z* 414, corresponding to the molecular formula $C_{29}H_{50}O$. This is also in close agreement with the literature values for β-sitostero (9). The ^{13}C-NMR of [2] also compared with the literature (10). The unequivocal identification of [2] was confirmed by direct TLC comparison with the reference commercial β-sitosterol (Sigma-Aldrich, USA, batch number 47C-0262). Co-chromatography of [2] and the reference sample gave identical $R_f$values and chromogenic reaction to spray reagents. β-sitosterol has been isolated from numerous species and different families *P. pinnata* (11).

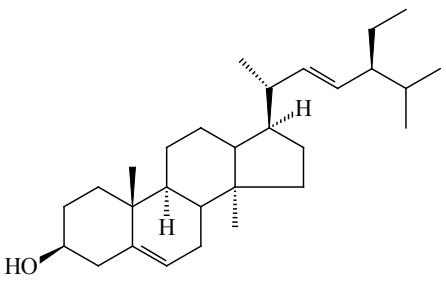
On crystallisation in methanol, [3] was obtained as white amorphous crystals with melting point 162-164°C (uncorrected). The low resolution EI-MS exhibited a maximum peak at *m/z* 412, corresponding to the molecular formula $C_{29}H_{50}O$. Other significant peaks in the MS spectrum were *m/z* 396, 351, 303, 255, 213, 159, 144, 107, 55. The ^{13}C-NMR also compared with the authentic sample obtained from Extrasynthèse (France) [Batch number, 0948460]. The ^{13}C-NMR spectrum [3] looked similar to that of β-sitosterol except the presence of additional peaks at 138.3 and 129.2 ppm, expected of methylene carbons, to those already present at 140.7 and 121.7 ppm (assigned as C-5 and C-6). These additional peaks were later assigned as C-22 and C-23 of the stigmasterol structure. The ^{1}H-NMR spectrum showed the presence of olefinic protons, by the downfield shift at 5.14 and 5.12 ppm by the H-22 and H-23 respectively.



1



2



3

The unequivocal identification of [3] was confirmed by direct TLC comparison (Pet. ether (40°-60°) - EtOAc [70:30]) with the reference commercial stigmasterol. Co-chromatography of [3] and the reference sample also gave identical R_f value (0.54) and chromogenic reactions. Stigmasterol has been isolated from numerous plant species.

Biological activity

It was noted that 2.0% of the total methanolic extract of *P. zeylanica* roots applied resulted in 100% ($p<0.01$) mortality against the larvae while 4.0% resulted in 100% ($p<0.01$) mortality against the nymphs and adult ticks. Petroleum spirit and ethyl acetate fractions made from the crude methanol extract showed a dose-dependant action on the tick stages with the larva being the most susceptible (Table 1).

Plumbagin was found to be most pronounced on the larvae and least pronounced on the adult tick with LD_{50} of 3.7×10^{-2} and 1.37 mg/ml respectively ($p<0.010$), while gamma benzene hexachloride (positive control) gave an LD_{50} values of 2.63×10^{-3} and 2.84×10^{-2} mg/ml against the larvae and adult ticks respectively, thus indicating a stronger action. β-sitosterol and stigmasterol isolated alongside plumbagin had no significant action on all stages of the tick (Table 2).

Tick infestation has been associated with the transmission of several important animal diseases involving bacteria, viruses, protozoa and rickettsia (12). The need therefore for the control of ticks to ensure a healthy livestock population cannot be over emphasized. However, the escalating cost of acaricides, the problem of resistance and the socio-economic factors which limit the use of chemical treatment of livestock owned by resource-poor farmers in Africa have made the search for alternative methods of tick control a priority. The potential of plants in animal husbandry has been highlighted (13, 14, 15).

Table 1. Acaricidal activity of extract/fractions expressed as the concentration (% w/v) that causes 100% mortality of tick stages

Extract/Fraction	Larva	Nymph	Adult
CMF	2.00	4.00	4.00
PF	0.20	4.00	4.00
EAF	0.20	2.00	4.00
AqF	—	—	—
BHC	0.00156	0.00652	0.0125

Key: CMF- crude methanolic extract; PF- petroleum ether fraction; EAF- ethyl acetate fraction; AqF- aqueous fraction, BHC- benzene hexachloride.

Table 2. Acaricidal activity (LD_{50}) of the isolated compounds on different stages of *Amblyoma variegatum*

Tick stage	Compound	LD_{50} (mg/ml)	% error
Larvae	Plumbagin	3.77×10^{-2}	0.3
	β-sitosterol	2.71	14.1
	stigmasterol	5.68	11.4
	BHC	2.63×10^{-3}	0.6
Nymph	Plumbagin	1.28×10^{-1}	1.5
	β-sitosterol	5.43	23.3
	stigmasterol	5.45	12.1
	BHC	1.81×10^{-2}	0.1
Adult	Plumbagin	1.38	16.0
	β-sitosterol	14.06	23
	stigmasterol	17.42	19
	BHC	2.84×10^{-2}	0.1

BHC- benzene hexachloride

In this study of the acaricidal constituents of *P. zeylanica*, a naphthaquinone plumbagin was found to be responsible for the activity. Previous phytochemical investigation of the plant revealed plumbagin as the major compound and some other related naphthoquinones which occur in trace amounts (Liu *et al*., 2006). Plumbagin has also been reported to be present in *Dyrophyton africanum, Drosera congolana, Diospyros spp*., *Diocophyllum tholonii* and *Sparaxis tricolor*, where it occurs with other naphthoquinones (16, 17). The acaricidal activity as demonstrated by high mortality of tick larvae exposed to the crude methanolic extract supports the folkloric use of the plant to control ticks.

Gamma benzene hexachloride (BHC), a known acaricide served as positive control and is used by majority of livestock farmers in Ghana in the management of tick infestation. This was found to have activity on all stages of the ticks with the LD_{50} on each stage lower than that of plumbagin. The LD_{50} of BHC on the larvae, nymphs and adult respectively were 2.68×10^{-3}, 1.81×10^{-2} and 2.84×10^{-2} mg/ml whilst those of plumbagin were 3.77×10^{-2}, 1.28×10^{-1} and 1.38×10^{-1} mg/ml. The above results infer that at each stage of the tick's life cycle, BHC is more potent than plumbagin as an acaricide. Microbiological investigation conducted by some workers on plumbagin has shown it to have a marked antimicrobial action on some *Staphyloccoci* and pathogenic fungi including *Trichophyton ferrugineum* (18). Hence using the plant for acaricidal purposes has an added advantage of controlling infections caused by these organisms and the potential to eliminate other diseases on the livestock since the concentration as acaricide (LD_{50} 3.78×10^{-2} mg/ml) falls above that as an antibiotic (MIC, 1.0 µg/ml).

CONCLUSIONS

Our findings have established that *P. zeylanica* extract have acaricidal actions against *A. variegatum* and that plumbagin is responsible for the acaricidal action. However, its potency as an acaricide is lower than gamma benzene hexachloride, a known chemical acaricide. The work finally supports the ethnobotanical use of *P. zeylanica* extract as acaricide in Ghana.

ACKNOWLEDGEMENT

We wish to thank the staff of the Animal Research Institute of the CSIR, Ghana for their assistance in this work.

ABBREVIATION

BHC	Benzene hexachloride
C-NMR	Carbon Nuclear Magnetic Resonance
HC-COBI	Heteronuclear correlation using a BIRD pulse
HMBC	Heteronuclear multiple bond coherence
IR	Infra red
LD_{50}	Median lethal dose
MIC	Minimum inhibitory concentration
PTLC	Preparative thin layer chromatography
TLC	Thin layer chromatography

REFERENCES

1. Sajid M.S., Iqbal Z., Khan M.N. and Muhammad, G. *In vitro* and *in vivo* Efficacies of Ivermectin and Cypermethrin against the Cattle Tick *Hyalomma anatolicum*. *Parasitol Res* **105**(4):1133–1138 (2009).
2. Lynen G., Zeman P., Bakuname C., Di Giulio G., Mtui P., Sanka P. and Jongejan F. Cattle Ticks of the Genera *Rhipicephalus* and *Amblyomma* of Economic Importance in Tanzania: Distribution Assessed with GIS Based on an Extensive Field Survey. *Exp Appl Acarol* **43**(4):303–319 (2007).
3. Puyvelde L.V., Geysen D., Ayobangira F., Hakizamungu E., Nshimiyimana A. and Kalisa A. Screening of Medicinal Plants of Rwanda for Acaricidal Activity. *J Ethnopharmacol* **13**: 209–215 (1985).
4. Coskun S., Girisgin O., Kurkcuoglu M., Malyer H., Girisgin A.O., Kirimer N., Baser K.H. Acaricidal Efficacy of *Origanum onites* L. Essential Oil Against *Rhipicephalus turanicus* (Ixodidae). *Parasitol Res.* **103**(2):259–261 (2008).
5. Shaw R.D. Culture of an Organophosphorus Resistant Strain of *Boophilus microplus* (Can.). *Bull Entomol Res* **56**:389–405 (1966).
6. Dyer R.J. *Applications of Absorption Spectroscopy of Organic Compounds*, Prentice - Hall Inc. Englewood Cliffs, N.J.
7. Heble M.R., Narayanaswamy S. and Chadba M.S. Tissue Differentiation and Plumbagin Synthesis in Variant Cell Strains of *Plumbago zeylanica* L. *in vitro*. *Plant Sci Lett*, **2**: 405–409 (1974).
8. Lee C.H. and Lee H.S. Acaricidal Activity and Function of Mite Indicator Using Plumbagin and its Derivatives Isolated from *Diospyros kaki* Thumb. Roots (Ebenaceae). *J Microbiol Biotechnol* **18**(2):314–321 (2008).
9. El Deeb K.S., Al-Haidari R.A., Mossa J.S. and Ateya A. Phytochemical and Pharmacological Studies of *Maytenus forsskaoliana*. *Saudi Pharmaceu J* **11**:184–191 (2003).
10. Kovganko N.V., Kashkan Z.N., Borisov E.V. and Batura E.V. ^{13}C-NMR Spectra of β-sitosterol Derivatives with Oxidized Rings A and B. *Chem Nat Comp* **35**:646–649 (1999).
11. Annan K., Adu F., Gbedema S.Y. Friedelin: A Bacterial Resistance Modulator from *Paullinia pinnata* L. *J Sci Technol* **29**(1):152–159 (2009).
12. Awumbila B. Acaricides in tick control in Ghana and methods of application. *Trop Animal Health Prod* **28**:19–20 (1996).
13. Cetin H., Cilek J.E., Aydin L. and Yanikoglu A. Acaricidal Effects of the Essential Oil of *Origanum minutiflorum* (Lamiaceae) against *Rhipicephalus turanicus* (Ixodidae). *Vet Parasitol*. **160**:359–361 (2009).
14. Apel M.A., Ribeiro V.L., Bordignon S.A., Henriques A.T. and von Poser G. Chemical Composition and Toxicity of the Essential Oils from *Cunila* species (Lamiaceae) on the Cattle Tick *Rhipicephalus (Boophilus) microplus*. *Parasitol Res* **105**(3):863–868 (2009).
15. Pirali-Kheirabadi K., Razzaghi-Abyaneh M. and Halajian A. Acaricidal Effect of *Pelargonium roseum* and E*ucalyptus globulus* Essential Oils Against Adult Stage of *Rhipicephalus (Boophilus) annulatus in vitro*. *Vet Parasitol*. **162**:346–349 (2009).
16. Liu Y., Deng F., Liu C., Meng Q.Y. and Gao Z.W. Determination of plumbagin in different partsof *Plumbago zeylanica* by RP-HPLC. *Zhongguo Zhong Yao Za Zhi* **31**(20):1684–1686 (2006).
17. Buckingham J. *Dictionary of Natural Products*, Vol. **5**., 1994, University Press, Cambridge.
18. Didry N., Dubreiuil L., Trotin F. and Pinkas M. Antimicrobial activity of aerial parts of *Drosera peltata* Smith on oral bacteria. *J Ethnopharmacol* **60**(1):91–96 (1998).

Pharmacognostical Evaluation of *Cicer arietinum* Linn.

Karnail Dalal Singh*, Sandeep Ahlawat and Arjun Patra

College of Pharmacy, IFTM, Moradabad- 244001, U.P, India

ABSTRACT

The Present study deals with the detailed pharmacognostical evaluation of *Cicer arietinum* Linn (Leguminosae). Morphoanatomy of the root part have been studied with the aim to aid pharmacognostic and taxonomic species identification. The physicochemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. arietinum* and can possibly help to differentiate the drug from its other species.

Keywords: Leguminosae, Pharmacognosy, *Cicer arietinum*.

Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** karnail_dalal@yahoo.co.in

INTRODUCTION

Cicer arietinum Linn belonging to family *Leguminosae* is an annual herb which is spread into Southern Europe, India, Egypt and Southern America. It is extensively cultivated in India mainly in Rajasthan, Hyderabad, Patiala, East Punjab, Haryana and Madhya Pradesh (1). It needs warm and moist climatic conditions to propagate. Its black gram is native of India but the white species commonly called Kabuli came to India in 18th century from European countries and area like Afghanistan etc. In India it is very often used as a crash diet and it is one of the most widely made recipes in India kitchen due to its good taste and nutritive values. Traditionally it is used as antibacterial, antifungal, antipyretic, antidiarrhoeal etc. The present investigation includes morphological, anatomical evaluation, determination of physicochemical constants and preliminary phytochemical screening of the hydroalcoholic (1:1) extract and its acetone, methanol fraction, ethanol, water, petroleum ether and chloroform extracts and TLC fingerprinting of different extracts of *Cicer arietinum* were also carried out.

MATERIALS AND METHODS

Collection and authentication of plant material

The Plant *Cicer arietinum* was collected during February to March from different region of Haryana and authenticated through NISCAIR, New Delhi and voucher specimen was preserved for further references. The roots were separated, washed under running tap water; air dried under shade, coarsely powdered and kept in airtight container until further use.

Macroscopic and microscopic analysis

The macroscopy and of the root were studied according to the method of Brain and turner (2). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (3). The micropowder analysis was done according to the method of Brain and Turner (4) and Kokate (5).

Physicochemical analysis

Physicochemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopoeia (6). Fluorescence analysis was carried out according to the method of Chase and Pratt (7), Kokoski *et al.* (8) and Evans (9).

Preliminary phytochemical screening

Preliminary phytochemical screening for organic and inorganic elements was carried out by using standard procedures described by Harborne (10) and Khandelwal (11).

Thin layer chromatography

Thin layer chromatography was performed using standard method of Burger, (12) and Janchen *et al.*, (13).

RESULTS AND DISCUSSION:

Macroscopic characters

The plant *Cicer arietinum* has a strong taproot system with 3 or 4 rows of lateral roots. The parenchymatous tissues of the root are rich in starch. All the peripheral tissues disappear at plant maturity, and are substituted by a layer of cork. The roots grow 1.5-2.0 m deep. *Cicer* roots bear rhizobium nodules. They are of the carotenoid type, branched with laterally flattened ramifications, sometimes forming a fanklike lobe. The outer surface is brownish in colour, rough with longitudinal wrinkles and wood part is light yellow in colour, hard and woody. Fracture is hard and short. The drug has slight characteristic taste and odour.

Microscopic characters:

Transverse section:

A single layer of epidermis, which consists of small tangentially elongated rectangular cells with brownish, thick-outer walls and a band of cortex, which consists of 9 to 10 rows of big parenchymatous cells with intercellular spaces (Fig. 1a). The inner part of cortex contains scattered groups of lignified fibres. Stellar region shows secondary growth. The Vascular bundle comprises of xylem and phloem. The secondary phloem consists of phloem fibres (Fig. 1b) and each phloem bundle is surrounded by a parenchymatous sheath containing calcium oxalate and starch grains. Medullary rays pass through both phloem and xylem. The secondary xylem was more developed and consists of vessel, tracheids, fibres and pitted parenchyma. Primary xylem was distinct on the inner side of the secondary xylem (Fig. 1c). The Medullary rays are uni-seriate or multi-seriate parenchymatous cells, narrow in the xylem region and wider in the phloem region (Fig. 1c). Medullary rays in the phloem region are non-lignified whereas lignified in the xylem region and starch grains (Fig. 1d) are present in few cells. Cambium consists of 2 to 3 rows of small irregular thin-walled cells (Fig. 1b) present above the secondary xylem and Pith consists of few rounds to oval thin walled parenchymatous cells (Fig. 2).

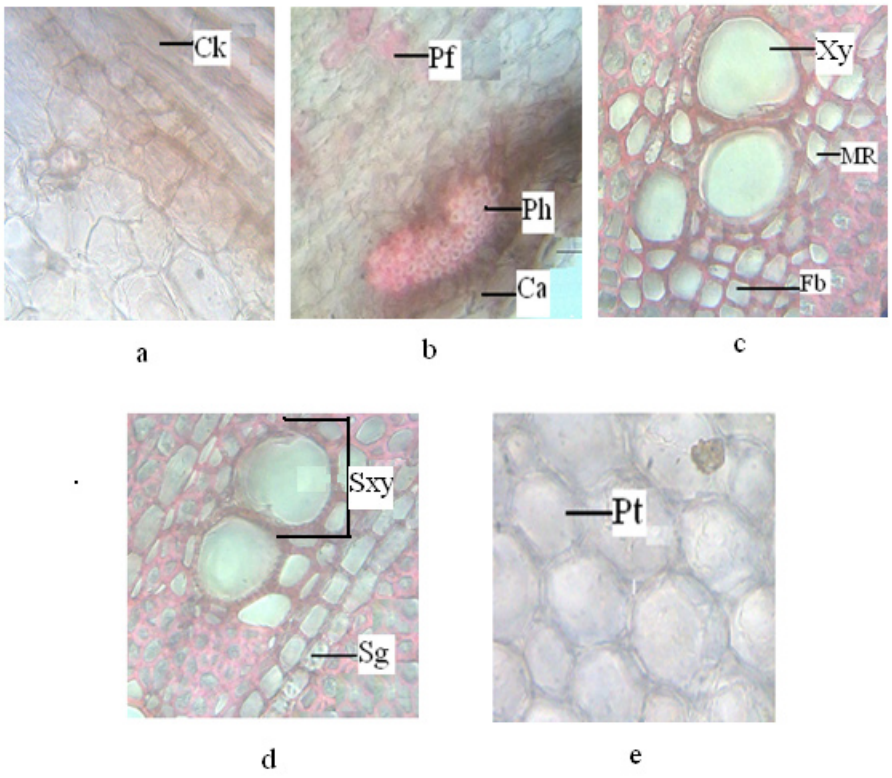


Figure 1. "Microscopy of root of *C. arietinum*"
a; Transverse section consists of Cortex (100x)
b; Transverse section contains Phloem fibre, Phloem, Cambium (100x)
c; Transverse section consists of Primary xylem, Medullary rays, Fibres (100x)
d; Transverse section consists of Secondary xylem, Starch grains (100x)
e; Transverse section consists of Pith (100x)
Abbreviations: Ca-Cambium, Ck-Cortex, Fb-Fibres, MR-Medullary rays, Pf-Phloem fibre, Ph-Phloem, Pt-Pith, Sxy-Secondary xylem, Sg-Starch grains, Xy-Primary xylem.

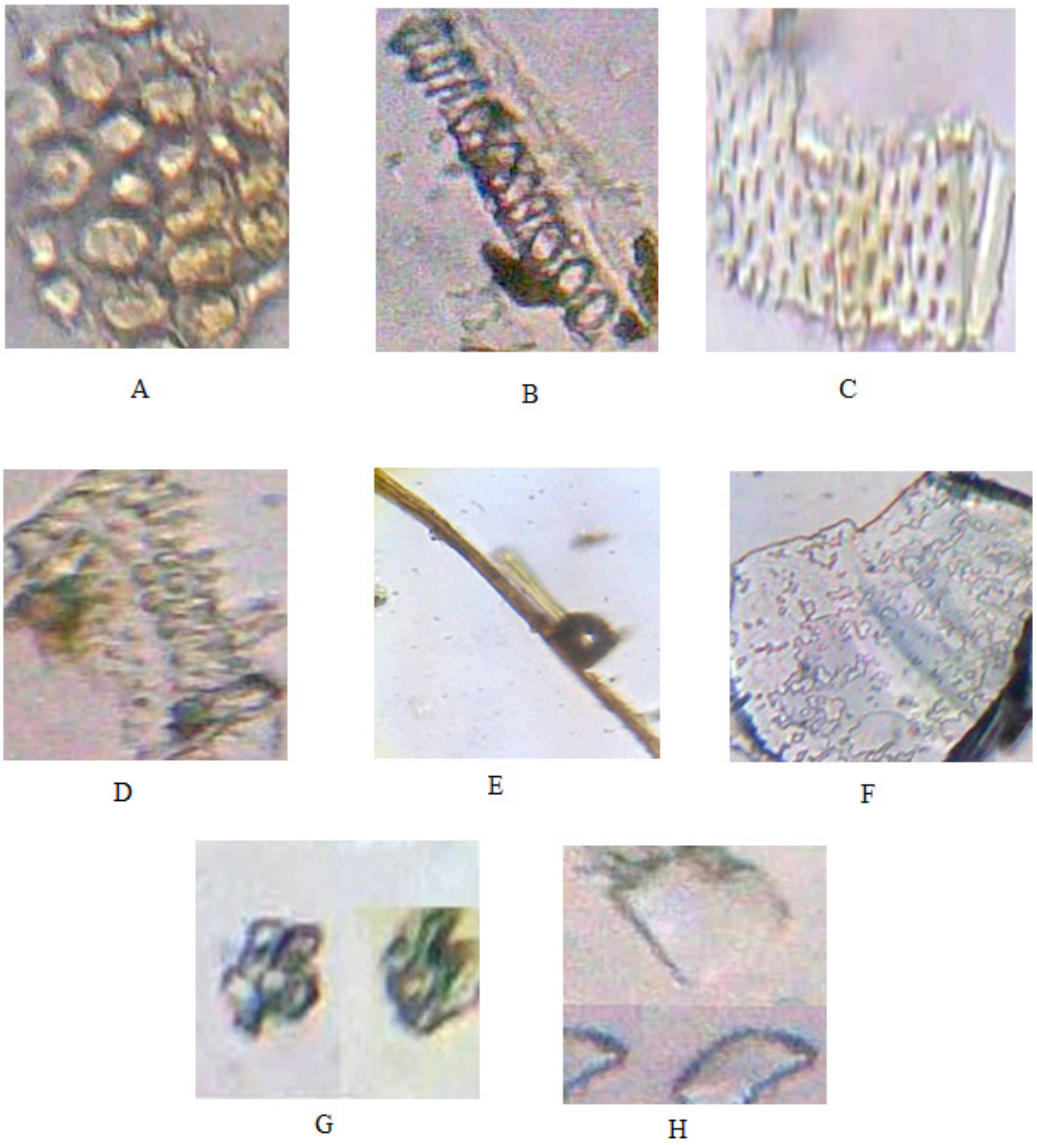


Figure 2. "Powder microscopy of *C. arietinum"*
A: Cork cells, B: Spiral vessel, C: Reticulate vessel, D: Border pitted vessel, E: Fibre, F: Trachids, G: Starch grain and H: calcium oxalate crystal.

Powder characteristic

The presence of cork cell, spiral vessel, reticulate vessels, bordered pits, fibres, tracheids, starch grain and crystals of calcium oxalate were observed (Fig. 2).

Preliminary phytochemical screening

Preliminary phytochemical screening for organic and inorganic elements was carried out by using standard procedures. The result of organic elements revealed the presence of carbohydrates, saponin, amino acid and protein (Table 1) and the result of inorganic elements shows the presence of iron, phosphate, sulphate, and chloride (Table 2).

Table 2. "Inorganic constituents of root powder of *C. arietinum*"

Elements	Results
Calcium	–
Magnesium	–
Sodium	–
Potassium	+
Iron	+
Sulphate	–
Phosphate	+
Chloride	+
Carbonate	–
Nitrates	–

(–) Not present, (+) present

Table 1. "Preliminary phytochemical screening of different extracts of C. arietinum root (organic elements)"

Phytoconstituents	Hydroalcoholic extract	Chloroform	extract	Acetone extract	Alcohol extract	Pet. Ether extract	Water extract	Methanolic fraction	Acetone fraction
Alkaloids									
Mayer's test	–	–	–	–	–	–	–	–	
Dragendroff's Test	–	–	–	–	–	–	–	–	
Wagner's test	–	–	–	–	–	–	–	–	
Hager's test	–	–	–	–	–	–	–	–	
Carbohydrates	+	+	+	+	–	+	+	+	
Amino acid	+	–	+	+	–	+	–	–	
Protein									
Biuret test	+	–	+	+	–	+	–	+	
Xanthoprotein test	+	–	+	+	–	+	–	+	
Million's test	+	–	+	+	–	+	–	+	
Saponin	–	–	–	–	–	+	–	–	
Tannin									
Ferric chloride test	–	–	–	–	–	–	–	–	
Lead acetate test	–	–	–	–	–	–	–	–	
Steroid and Triterpenoids									
Liebermann-Burchard test	–	–	–	–	–	–	–	–	
Salkowski's test	–	–	–	–	–	–	–	–	
Flavonoids	–	–	–	–	–	–	–	–	

(+) Sign indicates presence, (–) Sign indicates absence.

Physicochemical studies

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out in (Table 3). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble, acetone soluble, chloroform soluble and petroleum ether soluble extractive values have been tabulated in (Table 3). The results of fluorescence analysis of the root powder are presented in (Table 5).

Thin layer chromatography:

The TLC of hydroalcoholic, alcoholic and chloroform extracts of *Cicer arietinum* root was performed and the solvent systems were developed by running the plate on trial basis in different solvent systems in different ratio. The number of spots and their R_f values has been tabulated in (Table 4).

CONCLUSION

The present study on pharmacognostical evaluation of *C. arietinum* will provide useful information for

Table 3. "Physicochemical parameters of *C. arietinum*"

Parameters	Values (%w/w)
Alcohol soluble extractive	16.24
Water soluble extractive	7.36
Chloroform soluble extractive	3.28
Petroleum ether soluble extractive	0.72
Acetone soluble extractive	3.76
Moisture content (LOD)	13.33
Total ash	19.83
Acid insoluble ash	13.96
Water soluble ash	14.46

Table 4. " TLC of different extracts of root of *C. arietinum*"

Extracts	Solvent system	Number of spots and their Rf values
CACE	Toluene: Ether: Cyclohexane (2:1:1)	Three (0.23, 0.49,0.67)
CAHE	Toluene: Acetone: Methanol (2:1:1)	Two (0.58, 0.87)
CAAE	Toluene: Acetone: Methanol (2:1:1)	Two (0.56, 0.86)

CACE, chloroform extract; CAHE, hydroalcoholic extract; CAAE, alcoholic extract

Table 5. "Fluorescence analysis of the root powder of Cicer arietinum Linn"

Treatment	Day light	UV light	
		254 nm	365 nm
Powder as such	Light brown	Pinkish brown	Dark brown
Powder + Dil.HNO_3	Brown	Dark brown	Black
Powder + Dil. HCl (1N)	Light brown	Light Green	Dark Green
Powder + Conc.H_2SO_4	Dark brown	Black	Black
Powder + Ammonia	Greenish brown	Green	Black
Powder + Iodine solution.	Dark brown	Green	Black
Powder + $BaCl_2$ (10%)	Brown	Green	Black
Powder+ NaOH (Aq.40%)	Dark brown	Green	Black
Powder+$AgNO_3$ (10%)	Violet	Light Green	Black
Powder + $FeCl_3$ (5%)	Dark brown	Light Green	Dark Green
Powder+$MgSO_4$ (10%)	Light brown	Light brown	Dark Green
Powder+ $FeSO_4$ (10%)	Brown	Green	Black
Powder + $CaCl_2$ (5%)	Brown	Green	Black
Powder + Lead acetate (40%)	Light brown	Dark Green	Black
Powder + $CuSO_4$ (5%)	Greenish brown	Green	Black
Powder + Bromine water	Brown	Green	Black
Powder + Potassium Ferrocyanide (2%)	Brown	Green	Dark Green
Powder +Ammonium thiocyanate (5%)	Brown	Light Green	Dark Green
Powder +Ammonium molybdate (10%)	Brown	Green	Black
Powder + Sodium Cobalt nitrite (30%)	Dark brown	Dark Green	Black

its identification. Macro, micro and physiochemical standards discussed here can be considered as the identifying parameters to substantiate and authenticate the drug.

ACKNOWLEDGEMENT

The authors sincerely thank I.F.T.M, Moradabad for providing the necessary facilities to carry out the study.

REFERENCES

1. Raghanathan K. and Mitra R. *Pharmacognosy of Indigenous Drugs, I*, Central Council for Research in Ayurveda and Siddha. 2005; 214–224.
2. Brain K.R. and Turner T.D. *The Practical Evaluation of Phytopharmaceuticals*, Wright - Scientechnica, Bristol. 4–9 (1975a).
3. Johansen D.A. *Plant Microtechnique*, McGraw Hill, New York. 182 (1940).
4. Brain K.R. and Turner T.D. *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol. 36–45 (1975b).
5. Kokate C.K. *Practical Pharmacognosy*, 1st ed., Vallabh Prakashan, New Delhi. 15–30 (1986a).
6. *Indian Pharmacopoeia*, 4th ed., Vol. **II**, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi. A53–A54 (1996).
7. Chase C.R. and Pratt R.J. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. Am. Pharmacol. Assoc*. **38**: 32 (1949).
8. Kokoski J., Kokoski R. and Slama F.J. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J. Am. Pharmacol. Assoc*., **47**: 715 (1958).
9. Evans WC. *Pharmacognosy*, 15th ed. Edinburg London. 475, 101–105(2002).
10. Harborne J.B. Methods of extraction and isolation. In: *Phytochemical Methods*, Chapman & Hall, London. 60–66 (1998).
11. Khandelwal K.R. *Practical Pharmacognosy*, Nirali Prakashan. 149–157 (2008).
12. Burger K. Instrumental Thin Layer Chromatography/Planar Chromatography, Proceeding of the Instrumental Symposium, Brighton, UK. 33–44 (1989).
13. Janchen D. and Issaq H.J. *J. Liq. Chromatography*. **11**: 1941–1965 (1988).

Screening of Some Ghanaian Medicinal Plants for Phenolic Compounds and Radical Scavenging Activities

Asante Kojo Isaac[1]* Amuzu Nancy[1], Donkor Augustine[2] and Annan Kofi[3]

[1] Department of Botany, University of Ghana, Legon Ghana.
[2] Department of Chemistry, University of Ghana, Legon, Ghana.
[3] Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi Ghana

ABSTRACT

Extracts from a total of 37 medicinal plants from 15 families were screened for their phenolic compounds and antioxidant activities. Phenolic compounds identified were gallic, vanillic, syringic, caffeic, 2,5-dihydroxybenzoic, rosmarinic and p-coumaric acids. Gallic acid varied from 0.00 – 3.14 g/L, vanillic acid varied from 0.00 – 1.32 g/L, syringic acid varied from 0.00 – 3.40 g/L, caffeic acid varied from 0.00 – 2.04 g/L, 2,5-dihydroxybenzoic acid varied 0.00 – 2.64 g/L, rosmarinic acid varied from 0.00 – 1.90 g/L and p-coumaric acid varied from 0.00 – 2.23 g/L. DPPH radical scavenging action varied from 6.70 – 94.00 %. Extract from *Alchornea cordifolia* contained the highest amount of each of the seven different phenolic compounds. Extract of *Turraca heterophylla* did not contain any of the seven phenolic compounds. Extract of *Ocimum canum* exhibited the lowest level of DPPH radical scavenging activity of 6.70%. *Lantana camara* exhibited the highest radical scavenging activity of 94.40%. Multiple analyses suggest that 43.80 % of scavenging capacity of the herbal plants results from the contributions of the seven phenolic compounds. The study showed that scavenging effect of the plant extracts is not limited to phenolics but to other secondary metabolites. The study also showed that the Ghanaian medicinal plants used for the study can be good natural sources of phenolic compounds and antioxidants.

Keywords: Antioxidant, radical scavenger, phenols, 1,1-diphenyl-2-picryl hydrazyl
Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** E-mail: ikasante@ug.edu.gh. Tel: +233 224243792

INTRODUCTION

Phenolic compounds are plant secondary metabolites produced either from phenylalanine via the acetate pathway or from its precursor shikimic acid via the shikimate pathway (1). Being widely distributed and synthesized throughout the plant kingdom, with exception of fungi and algae, phenolic compounds are one of the most numerous classes of substances which are widely distributed and synthesized throughout the plant kingdom. Polyphenols have several important physiological functions in plants. They are essential for the regulation of growth and participate in the reproduction by attracting pollinating insects and also protect plants against UV radiation, harmful insects and infections (2). Their potentially versatile antioxidant activity is highly effective against diseases involved in antioxidative stress (3). Therefore polyphenols have been used in many antioxidant capacity assays before testing the properties of a biological system.

Of the polyphenol subclasses, phenolic acids and flavonoids are the most common and have often been described to be the most important as regarding the intake and potential effects in humans (4). As may be deduced by the large number of phenolic compounds and the discrepancy between the different subclasses, their chemical and biological properties vary greatly. Phenolic acids can be divided into those with a simple hydroxybenzoic acid structure (such as protocatechuic acid) and those with hydroxycinnamic acid structure (such as caffeic or rosmarinic acid) (5). So far, phenolic acids have been studied far less as compared to flavonoids. Hydroxybenzoic acids particularly have not been considered to be of great nutritional interest, as they are present in edible plants only in low amounts, with exception of some red fruits, berries, onions and cereals (6, 7). Hydroxycinnamic acids are commonly found in plants, and representatives of this class, such as caffeic, chlorogenic and ferulic acids, have been studied more.

Currently available synthetic antioxidants have been suspected to cause or prompt negative health effects. Therefore strong restrictions have been placed on their use and there is a trend to substitute with naturally occurring antioxidants. Synthetic antioxidants have also been found to show low solubility in body fluids (8). To overcome these challenges however, there has been a growing interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radicals induced injury. Natural antioxidants are commercially exploited as either antioxidant additives or nutritional supplements (9). In search for novel antioxidants, many plant species have been investigated (10, 11). However there is the need to search for more information concerning the antioxidant potential of plant species (8). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical spectrophotometrically.

In Ghana there is the widespread use of wild plants as medicines, however there are few reports of antioxidant activity and phenolic contents of these plants. In the present study we carried out an investigation of the free radical scavenging activity of selected Ghanaian medicinal plants. We also determined the contents of seven phenolic acids with antioxidant activity in the selected plants. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

METHODOLOGY

Standard compounds and reagents

The standard compounds used were obtained from Sigma Aldrich, UK and included gallic, vanillic, syringic, *p*-coumaric, caffeic, rosmarinic and 2,5-dihydroxybenzoic acids. The purity of all the standards used was 98% or higher. All other solvent and reagents used were of analytical or HPLC grade. Water used was purified using a Millipore RO 12 plus system (Millipore Corp., Bedford, MA, USA).

Plant materials

A total of 37 plants were collected between the months of March and April, 2008. They were authenticated at the Department of Botany, University of Ghana where voucher specimens are kept in the Herbarium. The leaves were air-dried for four days and milled into powder. About 1.0 g each of powdered sample was soaked in 2 ml methanol for 24 hrs, after which the contents were filtered and the filtrate kept in the fridge at 10°C for further analyses (Table 1).

Folin- Ciocalteau reagent (FCr)

One hundred grams of sodium tungstate and 25 g of sodium molybdate were dissolved in 750 mL distilled water. Fifty millilitres of 85% phosphoric acid and 100 mL of concentrated hydrochloric acid were added and the bottle swirled to mix. One hundred and fifty grams of lithium sulphate was then added followed by a few drops of bromine solution. The solution was heated to boil off the bromine. After cooling, the solution was stored in a refrigerator at 10°C.

Sodium carbonate solution (Na_2CO_3)

One hundred grams of sodium carbonate was dissolved in 400 mL of distilled water and brought to a boil. After cooling, a few crystals of sodium carbonate were added. The solution was filtered after 24 hrs and made up to 500 mL with distilled water.

Preparation of Calibration Curves

Stock solutions of the standard compounds were prepared by dissolving 0.50 g of each compound in 10 mL ethanol and diluting to 100 mL with distilled water. Serial dilutions of the stock were prepared to give concentration range of 0–600 mg/L.

From each calibration solution, 20 µL was pipetted into separate cuvettes, and to each, 1.58 mL water and 100 mL FCr was added. The contents were shaken to mix, and after 5 minutes, 300 µL of Na_2CO_3 solution was added and cuvettes were again shaken to mix. Solutions were placed in an oven at 40°C for 30 minutes. The absorbance of each solution was determined at 765nm against the blank. A graph of absorbance versus concentration was plotted for each of the standards, from which regression equations were developed.

Determination of phenols

Two milliliters of each of the 36 plant extracts were diluted to 20 mL with distilled water and 20 µL of the diluted samples were pipetted into separate cuvettes and to each 1.58 mL water and 100 µL FCr was added. After about 5 minutes, 300 µL of Na_2CO_3 was added and cuvettes were again shaken to mix. Solutions were placed in an oven at 40°C for 30 minutes. The absorbance of each solution was determined at 765 nm against the blank, and the values were recorded. The regression equations were applied to

Table 1. List of experimental plants, their families and voucher numbers

Species	Family	Voucher number
Alchornea cordifilia	Euphorbiaceae	UGB3708
Boerhavia diffusa	Nyctaginaceae	UGB3808
Cassia siamea	Caesalpiniaceae	UGB4308
Cinnamomum zeylanicum	Lauraceae	UGB4408
Clausena anisata	Rutaceae	UGB4508
Desmodium adscendens	Papilionaceae	UGB4608
Gymnema sylvestre	Asclepidiaceae	UGB4708
Heliotropium indicum	Boraginaceae	UGB5308
Jatropha gossypifolia	Euphorbiaceae	UGB5408
Lantana camara	Verbenaceae	UGB5508
Lippia multifolia (Attebubu)	Verbenaceae	UGB6008
Lippia multifolia (Kasoa)	Verbebaceae	UGB6108
Lippia multifolia (Mary)	Verbenaceae	UGB6208
Lippia multifolia (Kadjebi))	Verbenaceae	UGB6308
Mallotus oppositifolius	Euphorbiaceae	UGB6808
Moringa oleifera	Moringaceae	UGB6908
Ocimum basilicum (ABF017)	Lamiaceae	UGB7408
Ocimum basilicum (ABF042)	Lamiaceae	UGB7508
Ocimum basilicum	Lamiaceae	UGB7608
Ocimum canum (8)	Lamiaceae	UGB7708
Ocimum canum (14)	Lamiaceae	UGB7808
Ocimum gratissimum	Lamiaceae	UGB7908
Ocimum sanctum (1)	Lamiaceae	UGB8008
Ocimum sanctum (2)	Lamiaceae	UGB8108
Ocimum sanctum (9)	Lamiaceae	UGB8208
Ocimum sanctum (17)	Lamiaceae	UGB8308
Pegularia daemia	Asclepiadaceae	UGB8708
Phyllanthus amarus	Euphorbiaceae	UGB8808
Rauvolfia vomitoria	Apocynaceae	UGB8908
Secamone afzelii	Asclepiadaceae	UGB9008
Spondias mombin	Anacardiaceae	UGB9108
Turraca heterophylla	Meliaceae	UGB9208
Vernonia amygdalina (1)	Asteraceae	UGB9308
Vernonia amigdalina (2)	Asteraceae	UGB9408
Vinca roseus	Apocynaceae	UGB9608
Voacanga africana	Apocynaceae	UGB9708
Zanthoxylum xanthoxyloides	Rutaceae	UGB9908

determine the concentration of each of the standards in the samples (8).

1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay

Five millilitres methanolic DPPH solution was added to 60 µL of sample and incubated for 20 minutes in the dark. The absorbances of the solutions were then read at 517 nm using a UV-VIS spectrophotometer. A blank solution was prepared by adding 5mL of methanolic DPPH solution to 60µL of methanol (12). The DPPH scavenging effect was calculated using the formula:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} x\ 100\%$$

Where A_0 = Absorbance of the blank (control), and A_1 = Absorbance in the presence of the sample.

Table 2. Means (g/L) [SEM] of seven phenolic compounds and percent DPPH scavenging activities of the experimental plant samples (Each experiment was performed in triplicate)

Species	gallic acid	vanillic acid	syringic acid	2,5-dihydroxy benzoic acid	caffeic acid	rosmarinic acid	p- coumaric acid	DPPH scavenging activity (%)
Alchornea cordifilia	3.14±0.81	1.32±0.32	3.39±0.84	2.65±0.66	2.04±0.49	1.90±0.49	2.22±0.58	90.00±0.00
Boerhavia diffusa	0.14±0.47	0.11±0.23	0.21±0.47	0.13±0.37	0.14±0.30	0.11±0.27	0.08±0.34	53.00±0.00
Cassia siamea	0.85±0.47	0.40±0.20	0.97±0.50	0.73±0.37	0.59±0.27	0.54±0.27	0.59±0.34	78.90±0.00
Cinnamomum zeylanicum	2.33±0.47	0.10±0.20	2.54±0.50	1.97±0.37	1.53±0.27	1.42±0.27	1.65±0.34	88.53±0.03
Clausena anisata	0.87±0.43	0.41±0.20	0.99±0.47	0.74±0.00	0.61±0.30	0.55±0.27	0.60±0.34	14.50±0.06
Desmodium adscendens	0.14±0.00	0.12±0.00	0.22±0.00	0.13±0.40	0.15±0.00	0.11±0.00	0.09±0.00	82.90±0.00
Gymnema sylvestre	1.06±0.47	0.49±0.20	1.19±0.50	0.90±0.66	0.73±0.30	0.66±0.27	0.74±0.34	32.10±0.00
Heliotropium indicum	0.16±0.81	0.14±0.35	0.27±0.85	0.17±0.00	0.17±0.40	0.14±0.49	0.12±0.58	30.40±0.00
Jatropha gossypifolia	1.10±0.00	0.50±0.00	1.27±0.00	0.94±0.69	0.75±0.00	0.69±0.00	0.77±0.00	42.30±0.00
Lantana camara	0.35±0.78	0.20±0.32	0.46±0.84	0.30±0.00	0.28±0.52	0.23±0.46	0.23±0.58	94.40±0.00
Lippia multifolia (Attebubu)	0.51±0.00	0.27±0.00	0.61±0.00	0.45±0.40	0.38±0.00	0.33±0.00	0.35±0.00	70.30±0.00
Lippia multifolia (Kasoa)	0.25±0.47	0.16±0.17	0.34±0.50	0.22±0.00	0.22±0.27	0.18±0.27	0.16±0.34	59.00±0.00
Lippia multifolia (Mary)	0.53±0.00	0.27±0.00	0.63±0.00	0.46±0.40	0.38±0.00	0.34±0.00	0.36±0.00	36.30±0.00
Lippia multifolia (Kadjebi))	0.30±0.47	0.18±0.20	0.39±0.50	0.27±0.66	0.25±0.27	0.21±0.27	0.20±0.34	31.00±0.00
Mallotus oppositifolius	0.32±0.81	0.19±0.32	0.41±0.84	0.29±0.00	0.26±0.49	0.22±0.46	0.22±0.58	19.00±0.00
Moringa oleifera	0.21±0.00	0.14±0.00	0.29±0.00	0.19±0.00	0.19±0.00	0.15±0.00	0.13±0.00	8.73±0.03
Ocimum basilicum (ABF017)	0.28±0.00	0.17±0.00	0.36±0.00	0.25±0.00	0.23±0.00	0.19±0.00	0.18±0.00	63.20±0.00
Ocimum basilicum (ABF042)	0.10±0.00	0.10±0.00	0.18±0.00	0.01±0.00	0.12±0.00	0.09±0.00	0.06±0.00	53.70±0.00
Ocimum basilicum	0.86±0.00	0.41±0.20	0.97±4.20	0.74±3.35	0.60±2.51	0.54±2.37	0.69±2.86	52.00±0.06
Ocimum canum (8)	0.27±0.81	0.17±0.00	0.36±0.00	0.24±0.00	0.23±0.00	0.19±0.00	0.18±0.00	20.93±0.03
Ocimum canum (14)	0.39±0.00	0.22±0.00	0.49±0.84	0.34±0.66	0.31±0.00	0.26±0.46	0.27±0.55	6.70±0.00
Ocimum gratissimum	0.56±0.00	0.29±0.00	0.66±0.00	0.48±0.40	0.41±0.30	0.36±0.30	0.38±0.34	65.83±0.03
Ocimum sanctum (1)	2.20±0.00	0.94±0.00	2.39±0.00	1.86±0.00	1.45±0.00	1.34±0.00	1.55±0.00	85.60±0.03
Ocimum sanctum (2)	0.86±0.81	0.41±0.35	0.98±0.00	0.74±0.60	0.60±0.49	0.54±0.46	0.60±0.58	62.33±0.03
Ocimum sanctum (9)	0.89±0.47	0.42±0.20	1.01±0.84	0.76±0.40	0.62±0.27	0.56±0.27	0.62±0.34	69.73±0.03
Ocimum sanctum (17)	1.29±0.81	0.58±0.00	1.43±0.00	1.10±0.00	0.87±0.00	0.80±0.00	0.90±0.00	67.00±0.00
Pegularia daemia	0.27±0.00	0.17+0.00	0.36±0.00	0.24±0.00	0.23±0.00	0.19±0.00	0.18±0.00	9.30±0.00
Phyllanthus amarus	0.14±0.81	0.12±0.32	0.22±0.84	0.13±0.69	0.15±0.52	0.11±0.49	0.09±0.58	42.50±0.00
Rauvolfia vomitoria	0.56±0.81	0.29±0.20	0.66±0.50	0.48±0.37	0.41±0.30	0.36±0.27	0.39±0.30	22.60±0.00
Secamone afzelii	0.19±0.00	0.14±0.00	0.28±0.00	0.18±0.00	0.18±0.00	0.14±0.00	0.12±0.00	13.63±0.03
Spondias mombin	1.42±0.00	0.63±0.00	1.57±0.00	1.21.80±0.00	0.96±0.00	0.87±0.00	0.10±0.00	82.50±0.00
Turraca heterophylla	0.00±0.00	0.00±00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	6.90±0.00
Vernonia amygdalina (1)	0.43±0.00	0.23±0.00	0.53±0.00	0.38±0.00	0.33±0.00	0.29±0.00	0.29±0.00	57.70±0.00
Vernonia amigdalina (2)	0.19±0.78	0.14±0.32	0.28±0.87	0.18±0.66	0.18±0.52	0.14±0.49	0.12±0.58	46.70±0.00
Vinca roseus	0.44±0.43	0.24±0.17	0.54±0.50	0.39±0.00	0.34±0.27	0.29±0.27	0.30±0.34	33.30±0.00
Voacanga africana	0.80±0.78	0.39±0.32	0.93±0.84	0.69±0.66	0.57±0.52	0.51±0.46	0.56±0.58	57.70±0.00
Zanthoxylum xanthoxyloides	0.24±0.00	0.16±0.00	0.33±0.00	0.22±0.00	0.22±0.00	0.17±0.00	0.16±0.00	30.40±0.00
LSD (0.05)	2.35	0.95	2.48	1.96	1.47	1.39	1.68	0.06

Statistical analysis

Means, their standard errors and the least significant difference at P<0.05 were calculated using the SAS computer program.

RESULTS AND DISCUSSIONS

Phenol contents of the extracts

Seven phenolic compounds namely gallic, vanillic, syringic, caffeic, 2,5-dihydroxybenzoic, rosmarinic and p-coumaric acids were identified in the plant samples investigated (Table 2).

Syringic acid was the predominant phenolic compound in all the plants. It was highest in extracts of *A. cordifolia* (3.40 g/L) followed by *C. zeylanicum* (2.53 g/L), *O. sanctum* [1] (1.43 g/L). The highest concentration of gallic acid was observed in *A. cordifolia* (3.14 g/L), followed by *C. zeylanicum*, *G. sylvestre, J. gossypifolia* and *O. sanctum* (1). Similarly the highest concentration of vanillic acid was recorded for the extracts of *A. cordifolia* (1.33 g/L), followed by *C. zeylanicum* (0.10 g/L). The top five plant species with extracts of the highest concentrations of 2,5-dihydroxybenzoic acid were as follows: *A. cordifolia* (2.65 g/L), *C. zeylanicum* (1.97 g/L), *O. sanctum* [1] (1.86 g/L), *S. mombin* (1.21 g/L) and *O. sanctum* [17] (1.10 g/L). The pattern of variation for caffeic acid followed the same trend as 2,5-dihydroxybenzoic acid, the highest amount was found in extract of *A. cordifolia* (2.04 g/L), followed by *C. zeylanicum* (1.53 g/L). Extract of *A. cordifolia* recorded the highest amount of rosmarinic acid and p-coumaric acid (1.90 g/L and 2.23 g/L, respectively) followed by *C. zeylanicum* (1.42 g/L and 1.65 g/L respectively) and *O. sanctum* [1] 1.34 g/L and 1.55 g/L respectively). Extract of *Turraca heterophylla* did not contain any of the seven phenolic compounds. Previous studies (8) have shown that phenol compounds in extracts of Iranian medicinal plants varied from 0.02 - 0.29 g/L. This amount is lower than the highest values observed in the present study in extract of *A. cordifolia*. The observed difference could be explained on the basis of different climatic and soil conditions.

DPPH scavenging activity

Antioxidant activities of the plants are shown in Table 2. They ranged from 6.7–94.0%. Multiple analyses suggest that 43.8 % of scavenging capacity of the herbal plants results from the contributions of the seven phenolic compounds. It can also be concluded that scavenging effects of the plant extracts is not limited to phenolics. Scavenging activity may be attributed to the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids, tannins and minerals among others, that in this case contributed 56.2 % of the antioxidant capacity. In this study, extracts of *T. heterophylla* contained no amount of any of the phenolic compounds but exhibited 6.9% of antioxidant activity. In a study of antioxidant activity, phenol and flavonoid contents of some Iranian medicinal plants, Pourmorad *et al* (8) observed a DPPH radical scavenging activity of 94.3% in extracts of *Mallilotus officinalis*. In this study extracts of *L. camara* and *A. cordifolia* exhibited antioxidant activities of 94.0% and 90.0%, respectively.

Free radicals are by-products of biological reactions or they are generated from exogenous factors. There is documentation on the involvement of free radicals in the pathogenesis of a large number of diseases (8). A potent scavenger of free radicals may thus serve as a possible preventive intervention for the diseases (12).

CONCLUSIONS

The result of the present study showed that the extract of *A. cordifolia* had the highest amount of the seven phenolic compounds: gallic acid, vanillic acid, syringic acid, caffeic acid, 2,5-dihydroxybenzoic acid, rosmarinic acid and p-coumaric acid and therefore can be a very good natural source. The 90.0% scavenging property of *A. cordifolia* may be due to hydroxyl groups existing in these phenolic compounds chemical structure that can provide the necessary component as a radical scavenger. Extract of *L. camara* exhibited the greatest antioxidant activity of 94.0% though it did not contain as much levels of the seven phenolic compounds as extract of *A. cordifolia*. In this case other factors might be responsible for the scavenging activity in extract of *L. camara*. This study has shown that the Ghanaian medicinal plants studied can serve as potential sources for both phenolic compounds and antioxidants.

ACKNOWLEDGEMENT

We would like to thank the technical staff of the Department of Botany, University of Ghana, Legon, for their assistance in this project.

REFERENCES

1. Harborne J.B. Methods in Plant Biochemistry I: Plant Phenolics. Academic Press, London, UK, 1989.
2. Korkina L.G. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol 53, 15–25 (2007).
3. Lee C.Y., Jenner A.M. and Halliwell B. Rapid preparation of human urine and plasma samples for analysis of F2-isoprostanes by Gas

Chromatography–Mass Spectrometry. Biochem Biophys Res Commun 320: 696–702 (2004).

4. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56: 317–333 (1998).
5. Manach C., Scalbert A., Morand C., Remesy C. and Jimenez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–47 (2004).
6. Mattila P., Pihlava J.M. and Hellström J. Content of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. J Agric Food Chem 53: 8290–5 (2005).
7. Mattila P., Hellström. J. and Törrönen R. Phenolic acids in fruits, berries and beverages. J Agric Food Chem 54: 7193–9 (2006).
8. Pourmorad F., Hoseinimehr S.J. and Shahabimajd N. Antioxidant activity, phenol flavonoid contents of some selected Iranian medicinal plants. Afr J Biotech 5: 1142–5 (2006).
9. Schuler P. Natural antioxidants exploited commercially. In Food Antioxidants, Hudson, B.J.F. (ed.) Elsevier, London, pp 99–170, 1990.
10. Chu Y. Flavonoid content of several vegetables and their antioxidant activity. J Sci Food and Agricul 80: 561–6 (2000).
11. Oke J.M. and Hamburger M.O. Screening of some Nigerian medicinal plants for antioxidant activity using 2,2-diphenyl-picryl hydrazyl radical. Afr J Biomed Res 5:77–9 (2002).
12. Gyamfi M.A., Yonamine M. and Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana, Thoningia sanguinea on experimentally-induced liver injuries. Gen Pharmacol 32: 661–7 (1999).

Ethylacetate Extract Of Annona Squamosa Seeds Containing Anti-Head Lice Activity

Aditya V. Ashok*, Srinivas A., Ravitej K., Gopinadh V.

A. Srinivas, department of pharmaceutical chemistry, Hindu college of pharmacy, Guntur Andhrapradesh, India, srinu.annadatha@gmail.com.
K. Ravitej, department of pharmaceutical chemistry, Hindu college of pharmacy, Guntur Andhrapradesh, India, ravitej@gmail.com
V. Gopinadh, department of pharmaceutical chemistry, Hindu college of pharmacy, Guntur Andhrapradesh, India, gopinadh@gmail.com
V. Ashok Aditya*, department of pharmaceutical chemistry, Hindu college of pharmacy, Guntur Andhrapradesh, India, adityaashok5@gmail.com

ABSTRACT

The present study focused on the separation and identification of the active compounds against head lice from the ethyl acetate extract of *Annona squamosa* L seed. Chromatographic and spectroscopic techniques revealed that two major compounds of the hexane seed extract were oleic acid and triglyceride with one oleate ester. The yields of these compounds were 13.88% and 7.70% dry weight, respectively. The compounds were tested *in vitro* against head lice, comparing to the crude ethyl acetate extract of the seed. The triglyceride with one oleate ester and the crude ethyl acetate extract diluted with coconut oil 1:1. These compounds were found to kill all tested head lice in 10 and 31 minutes, respectively. The triglyceride ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material *A. squamosa* seed and its extract. This finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant.

Keywords: *Annona Squamosa*, anti-head lice activity, silver-ion column chromatography.

Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** E-mail: adityaashok5@gmail.com Mob: 09848076107

INTRODUCTION

Annona squamosa L. (Custard apple) is a plant belonging to the family Annonaceae. It is found in all parts of India especially in southern parts of India, Sugar apple, Sweet sop. The human head louse (*Pediculus humanuscapitis*) is a small insect causing a public health problem, especially in poor sanitary conditions. Farmers in Vietnam use seed oil to control rice leafhoppers and plant hoppers (1) .In Thailand, research has shown the anti-head lice activity of *A. squamosa*. Puapatanakul (6) reported that the extract of custard apple seeds in coconut oil at the ratio of 1:2 can kill 98% of head lice within two hours, while the leaf extract shows less potency. Gritsanapan *et al* (4) found that the petroleum ether extract of the leaves and seeds dissolved in coconut oil at a ratio of 1:1, kill 90% of head lice *in vitro* by 53 and 26 minutes, respectively. A 20% cream (oil/water) preparation of petroleum ether extract of custard apple seeds can kill 93% of head lice. Within 3 hours however, it is easier to control the quality and stability of the preparation by quantitative analysis of the active chemical components. The present study, therefore, is focused on the isolation and identification of the components in the seeds of *A.squamosa* having anti-head lice effect present.

MATERIALS AND METHODS

Preparation of plant seed crude powder:

A.squamosa seeds were collected from a village of Guntur district, Andhrapradesh, India in December 2008.Seeds were removed manually from these fruits, washed in water and dried in a hot air oven at 55°C for 24 hours. The dried seeds were ground in an electric mill.

Extraction and separation of major compounds:

The powdered seeds of *A. squamosa* (1 kg) were placed in soxhlet extractor. The solvent ethyl acetate is used

for successive extraction. Extraction was done for 48 hours until the solvent extracted have no color. After extraction the mixture was filtered and distillation was carried out with rotary evaporator to remove the solvent and evaporated in a hot water bath until a constant weight (280.5g) was obtained. The extract (75 g) was separated using silver-ion column chromatography with dichloromethane/methanol (90/10, v/v) was used as an eluent. Fifty milliliter fractions were collected and the fractions with the same silver-ion TLC pattern (hexane/ diethyl ether (90/10, v/v) were combined. The fractions containing two major spots (*Rf* 0.20 and 0.72) were eluted in 100% CH_2Cl_2 fractions.

To isolate pure compounds, the fractions containing major compounds were combined and concentrated. The mixture was further fractionated using silver-ion column chromatography. Isocratic elution by hexane: diethyl ether (90/10, v/v) was performed (approximately 25ml per fraction). The fractions with the same TLC pattern were combined to yield five fractions. The second and fourth fractions gave compound AS1 (38.7 g) and compound AS2 (22.6 g), respectively. Compounds AS1 and AS2 were purified to give pure compounds.

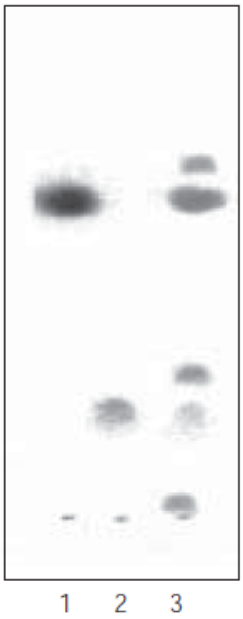
Testing for anti-head lice activity of pure compounds and crude extract:

The ethyl acetate extract and the two major pure compounds were tested for anti-head lice activity. The extract and pure compounds were separately dissolved in coconut oil at dilutions of 1:1to 1:8 (w:w). The same amount of each solution (0.05 ml) was put in a Petri dish and spread in a thin layer over a 2 cm^2 area. Seven equal sized head lice collected from school girls' hair were placed in the Petri dish containing solutions of the extract and the two major pure compounds. Non-moving head lice, which were determined as dead lice, were counted every 5 minutes until all the lice were dead. A commercial anti-head lice cream, Hexin, which is gamma benzene hexa chloride (1% w/w) and coconut oil were used as a positive and negative controls, respectively.

RESULTS

Compound AS1 was pale yellow oil, yielded 13.88% w/w of dried seeds. The silver-ion TLC hexane/diethyl ether (90/10, v/v) had an *Rf* value of 0.20 (Fig 1). The EI mass spectrum had a molecular ion peak at *m/z* 283.2 [M+1] and a prominent peak at *m/z* 264.3.

The IR spectrum of compound AS1 revealed absorption peaks at 3000-2930 (O-H stretch), 2850 (C-H stretch), and 1700 (C=O stretch, carboxylic) cm^{-1}.



1 = AS1, 2 = AS2, 3 = ethyl acetate crude extract.
Figure 1. Silver-Ion TLC chromatogram of AS1, AS2 and ethyl acetate crude extract

The ^1H NMR spectrum of compound AS1indicated the presence of one methyl proton at δ 0.90 (3H, t, H-18); a methylene proton group at δ 1.26 (20H, m, H-4-7 and H-12-17); two methylene proton groups at δ1.65 (2H, m, H-3);four methylene proton groups at δ 2.00 (4H, m,H-8, 11); two methylene proton groups at δ 2.35(2H, t, H-2); two olefinic methane proton group sat δ 5.35 (2H, m, H-9,10) and the broad peak of a hydroxyl proton at δ 10.15 (1H).

The ^{13}C NMR spectrum and Distortion less Enhancement by Polarization Transfer (DEPT) exhibited 16 carbon resonances, revealing the presence of thirteen methylene carbons, one methyl carbon, two olefinic methine carbons and one carbonyl carbon.

These spectral data suggested that compoundAS1 was a fatty acid. Comparing the NMR spectra of compound AS1 with Aldrich Library (1993) of 13C and 1H FT NMR spectra, confirmed the molecular structure of AS1 was an oleic acid (Fig 2).

Compound AS2 was also pale yellow oil, yielded 7.70% w/w of dried seeds. The *Rf* value(silver-ion TLC hexane/diethyl ether (90/10, v/v)) was 0.72 (Fig 1).The ^1H NMR spectrum looked similar to the AS1 spectrum, with additional signals at δ4.15 and 4.30.

The IR spectrum of compound AS2 showed bands at 2925 (C-H stretch) and 1746 (C=O stretch, ester) cm^{-1}.

After comparing the NMR spectra of compoundAS2 with Aldrich Library (1913) of ^{13}C and^1H FT NMR spectra, compound AS2 was felt to be a triglyceride with one oleate ester (Fig 2).

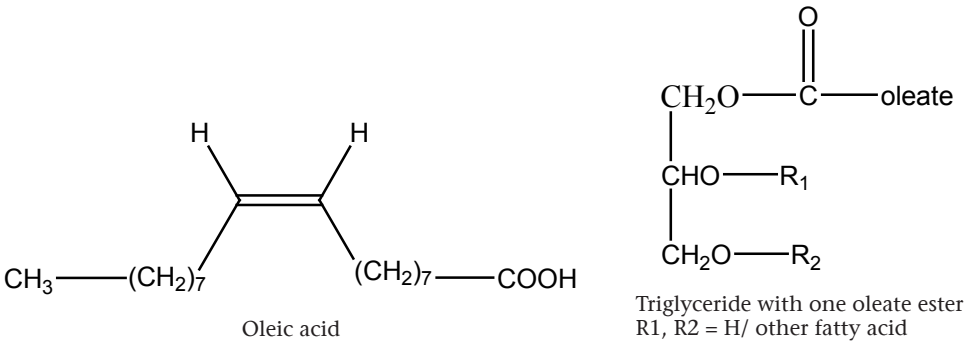


Figure 2. Structures of separated compounds from *Annona squamosa* seed

Test sample	Dilution (w:w)	Killing time (min)
Ethyl acetate crude	1:1	31.67±4.04
extract	1:2	34.33±4.04
	1:4	41.00±3.61
	1:8	55.00±5.00
leic acid (AS1)	1:1	47.33±3.06
	1:2	54.67±5.51
	1:4	59.00±6.56
	1:8	61.33±4.16
Triglyceride with one		
oleate ester (AS2)	1:1	10.00±1.00
	1:2	12.00±2.00
	1:4	16.00±1.00
	1:8	22.33±2.52
Coconut oil (-ve control)	Not diluted	>180
Hexin (+ve control)	Not diluted	>180

The results of anti-lice activity of ethyl acetate extract of seed of *Annona squamosa* is given

DISCUSSION

The purified form of ethyl acetate which contains Triglyceride with one oleate ester (AS2) shows maximum killing effect of lice at 1:8 (w:w) dilution with coconut oil with in less time , 22.33±2.52.The purified form of ethyl acetate crude extract in coconut oil (1:1) was significantly more active against head lice than gamma benzene hexachloride 1% cream and the hexane crude extract. These data are supported by previous reports of Gritsanapan *et al* (4). This result is useful for the standardization of *Annona squamosa* seed and its extract. The active compound may be used for the qualitative assessment of the chemical stability of the custard apple cream preparation.

ACKNOWLEDGEMENTS

We are thankful to my parents for providing financial support, to my management and HOD of pharmaceutical chemistry, Hindu college of pharmacy for providing necessary facilities for this project work.

REFERENCES

1. Brady N.C., Khush G.S. and Heinrichs E.A. (1978). Visit of the IRRI team to the Socialist Republic of Vietnam April 13-May 19. *IRRI Annual Report*: **49**.
2. Aldrich Chemical Co, Inc. *The Aldrich Library of 13Cand 1H FT NMR Spectra*. 1st ed, 1993.
3. Boonyaprapasara N., Chokchaicharorenporn O. and eds.Maipuenbaan Samunprai. Vol. **2**. Bangkok: Faculty of Pharmacy, Mahidol University, 1998: 454(in Thai).
4. Gritsanapan W., Somanabandhu A., Titirungruang C. and Lertchaiporn M.. A study on the anti-parasitic activities and chemical constituents of extracts from the leaves and seeds of custard apple (*Annona squamosa* Linn.). *Proceedings of Third NRCTJSPS Joint Seminar*, 1996: 209–15.
5. McCage C.M., Ward S.M., Paling C.A., Fisher D.A., Flynn P.J. and McLaughlin. Development of a paw paws herbal shampoo for the removal of head lice. *Phytomedicine* 2002; **9**: 743–8.
6. Puapatanakul O.. *Clinical studies of Annona squamosa seeds and leaves for the treatment of head lice*.1980: **37** pp (in Thai).
7. Tiangda C., Gritsanapan W., Sookvanichsilp N. and Limchalearn A.. Anti head-lice activity of *Annona squamosa* seed extract. *Southeast Asian Journal of Tropical Medicine and Public Health* 2000; **31**(suppl 1): 174–7.

Pharmacognostical studies and antibacterial activity of the leaves of *Murraya koenigii*

Jaju Shivkanya[1], Pahwa Shilpa[1], Kumari Sangita[1], Fuloria Neeraj[2]

1. Institute of Pharm. Tech, N.I.E.T., 19, Knowledge Park-II, Gr.Noida, U.P. (India)
2. R. V. Northland, GR. Noida-II, DADRI, U.P. (India)

ABSTRACT

The present study deals with the macro and microscopical studies as well as antibacterial studies of *Murraya koenigii* Linn.leaf. Some distinct characters were observed while studying their transverse sections. Macroscopically, the leaf's shape was lanceolate measuring 4.9 cm long and 1.8 cm broad with a reticulate venation
Microscopically, the midrib and laminar region showed a distinct epidermis. The collenchyma was thick walled followed by loosely arranged parenchymatous cells containing oil and starch grains. Physiochemical and preliminary phytochemical studies of the leaf were also carried out. The antibacterial studies confirmed that the methanolic extract was quite effective for *S.typhi* and *E.coli* at 100 µg/ ml and 200 µg/ ml respectively. The present study might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in the present scenario lacking regulatory laws to control quality of herbal drugs and also to find out the antibacterial activity.

Keywords: antibacterial, leaf, macroscopical, methanolic extract, microscopical, *Murraya koenigii*.

Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** E-mail: shilpapahwa@yahoo.co.in

INTRODUCTION

Murraya koenigii (L) is an aromatic, more or less deciduous shrub or a small tree upto 6 mm in height found throughout India up to an altitude of 1,500 m[1]. The plant grows best in tropical and subtropical climates, in sunny to semi shaded locations. They are very frosts sensitive. It is traditionally used as as an antiemetic, antidiarrhoeal, ferifuge and blood purifirer[4,7]. It is used as a flavoring agent in Indian dishes (Curry). *M.koenigii* (Rutaceae) is commonly known as Methi neem.

No report is available on micro-morphological as well as antibacterial activity of this drug, hence the present study was undertaken to explore pharmacognostical investigation of *Murraya koenigii* leaf.

MATERIAL AND METHODS

The plant specimens for the study were collected from the botanical garden of N.I.E.T, Gr.Noida, UP, India. It was identified and authenticated by Dr.Anjula Pande, Taxonomist, Pusa (India). Care was taken to select healthy fully grown plant. The samples collected were dried under the sunlight.

Macroscopy

The leaves were examined for size, shape, color, odour, taste, venation, etc.

Micoscopy

The outer epidermal membrane layer was cleared in chloral hydrate and warmed for half an hour. It was then stained with phloroglucinol and conc.HCl and mounted with glycerine and observed under a compound microscope[6]. The presence/ absence of the following were observed: Epidermal cell, Stomata (Types and distribution) and epidermal hair (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and midrib as well as small quantity of powdered leaves were also clearly mounted and observed.

Photomicrograph

Microscopic descriptions of selected tissues were photographed using Radical RXLR-3T 28378

Phytochemical Examination

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites.[2,5]

Quantitative Investigation

Quantitative leaf microscopy to determine Palisade ratio, Stomata number, Stomata index, Vein islet number and vein termination number were carried out on epidermal strips.

Physical Evaluation

The powdered leaves were determined for Total ash, acid-insoluble, water soluble ash value, sulphated ash value and water soluble and alcohol soluble extractive value.[10]

Fluorescence study

It is an essential parameter for first line standardization of crude drug. The crude drug was subjected to this study and its fluorescence pattern was noted. The powdered material was treated separately with different reagents and exposed to visible, UV light (Short and long) to study their fluorescence behaviour.[9]

Antibacterial Activity[2,8]

Preparation of Methanolic Extract

Hundred grams of the dried leaves of *Murraya koenigii* were boiled in a soxhlet with 300 ml of methanol for 24 h.The entire extract of *Murraya koenigii* leaves was evaporated to dryness in a rotary evaporator.

Microorganisms

Test micro-organisms (*S.aureus, E.coli & S.typhi*) were obtained from the laboratory stock culture. The test micro-organisms were cultured on nutrient agar slants at 37°C for 18 h. The stock cultures were maintained on nutrient agar slants at 4°C.

Antibacterial Activity

The antibacterial activity of the crude extract was determined following the method described in IP, 1996. (Table 1)

RESULTS AND DISCUSSION:

Macroscopy

The following characteristics of fresh leaves were noted:

Colour : Green
Odour : Characteristic
Taste : Characteristic
Shape : Lanceolate
Size : 4.9cm long, 1.8 cm broad
Extra feature : 24 leaflet having reticulate venation

Microscopy

The T.S.of leaf of *M.Koenigii* showed the following structures:

Table 1.Antibacterial Activity

S.No.	Standards (µg/ ml)	Test (µg/ ml)(Methanolic extracts of leaf)
1	10	5
2		10
3	20	50
4		100
5		200

CONTROL: Distilled water with 0.1% Tween 80

MIDRIB PORTION

Epidermis-Single layered, parenchymatous, uniseriate, unicellular covering with trichome

Collenchyma- Below epidermis is a compactly thick walled collenchymatous cells followed by loosely arranged parenchymatous cells containing oils and starch grains.

Vascular Bundle- It consists of a xylem vessels and parenchymatous phloem cells followed by a multilayered thick walled parenchymatous cells containing cellulose.

LAMINA

Upper Epidermis- Single layered polygonal straight cells covered with cuticle.

Mesophyll-Upper palisade cells were single layered, elongated, compactly arranged followed by spongy

Table 2. Phytochemical Investigations

S.No.	Secondary Metabolites	Results
1	Volatile oils	+
2	Carbohydrates	+
3	Glycoside	+ (Cardiac glycoside, Anthraquinone, coumarin glycoside
4	Proteins	+
5	Vitamins	+ (Vitamin A)

+ = the constituent is present

Table 3. Quantitative Investigation:

Parameters	Result
Stomatal number	Upper Epidermis-3 per sq mm Lower epidermis- 2 per sq mm
Stomatal index	Both surfaces-40-60
Palisade ratio	1:10
Vein islet number	18
Vein Termination Number	8

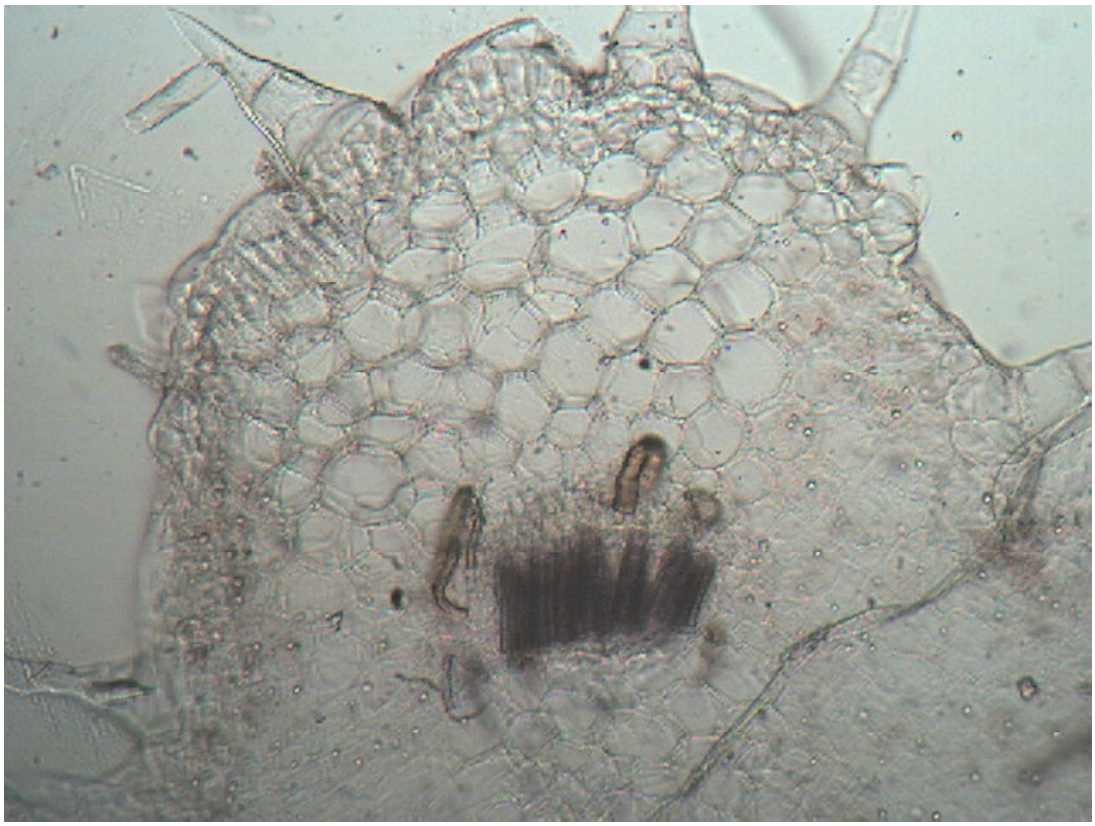


Figure 1. T.S.of leaf (Midrib Portion)

Figure 2. Vascular Bundles (Xylem Vessels)

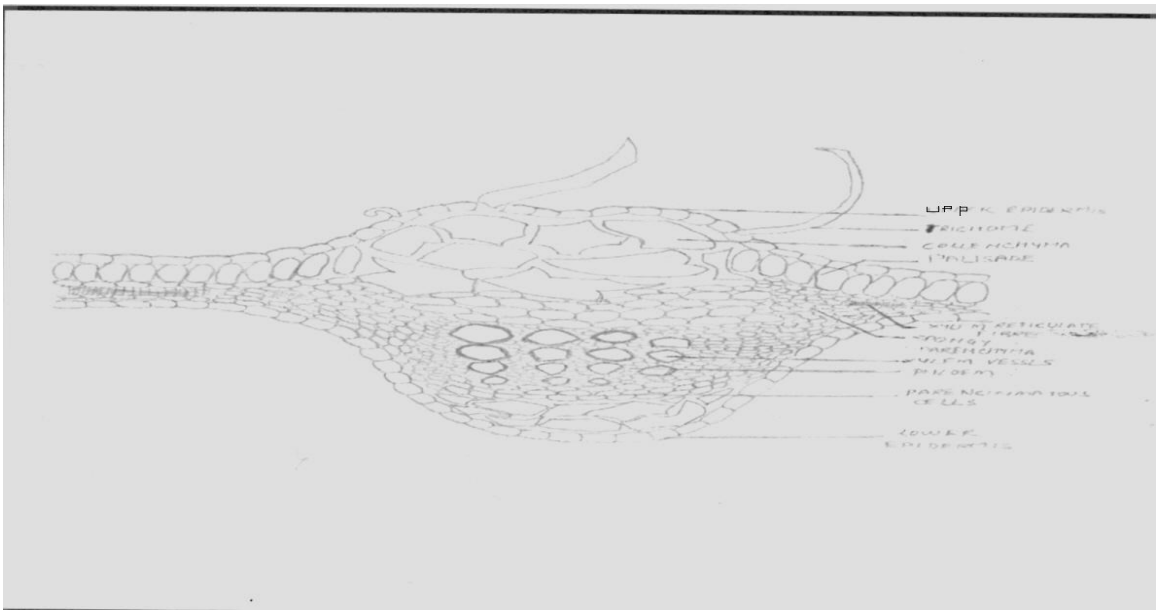


Figure 3. Complete T.S of Leaf (Both lamina and midrib portion)

parenchyma, thin walled, closely arranged intracellular spaces.In between these cells xylem reticulate fibres were seen.

Lower Epidermis- Single layered, polygonal, straight cells were found.

Phytochemical Investigations:

The following phytochemical constituents were seen as shown in Table 2

Quantitative investigation:

The results for Palisade ratio, Stomatal number, Stomatal index, Vein islet number and vein termination number

Table 4. Physical Evaluation:Ash Value

Parameters	Result
Total ash value	4.86% w/w
Acid insoluble ash value	0.37% w/w
Water soluble ash value	1.47% w/w
Sulphated ash value	9.5% w/w

Extractive Value

Parameters	Result
Alcohol soluble extractive value	3.04 w/w
Water soluble extractive value	0.26 w/w

Table 5. Fluorescence Analysis of Powder

Treatment	Visible (400–800 nm)	U.V.short (254 nm)	U.V.long (365 nm)
As such	Yellow	Yellow	Brownish yellow
Methanol	Yellow	Yellow	Brownish yellow
1N NaOH	Greenish yellow	Yellow	Dark yellow
Methanol + NaOH (1:1)	Yellow	Light Yellow	Dark yellow
Ethanol	Yellow	Black	Dark yellow
Conc.HCl	Brownish yellow	Light yellow	Brownish yellow
H_2SO_4 (66 %)	Yellow	Black	Black
Conc H_2SO_4	Black	Black	Black
Nitric acid	Dark brown	Yellow	Brown

Table 6. Results of Antibacterial Activity

Sample	Conc	*S.typhi*	*S.aureus*	*E.coli*
Standard	10 µg/ ml	3 mm	4 mm	6 mm
(Amoxicillin)	20 µg/ ml	4 mm	4 mm	8 mm
Test	5 µg/ ml	16 mm	20 mm	17 mm
(Methanolic	10 µg/ ml	19 mm		19 mm
Extract)	50 µg/ ml	21 mm		17 mm
	100 µg/ ml	17 mm		18 mm
	200 µg/ ml			

etc carried out on epidermal strips has been shown in Table 3

Physical Evaluation:

The following were the results as shown in Table 4

Fluorescence:

The powder showed the following results when exposed to different reagents and to visible and UV light.

Antibacterial Activity:

In the antibacterial activity screening the extract inhibited the growth of *S.typhi* and *E.coli* with a moderate zone of inhibition. (Table 6).The zone of inhibition of the methanolic extracts were the same (100 µg/ ml) against *S.typhi* and *E.coli*.

DISCUSSION:

Standardization of herbal drugs means a systemic approach to quality control. Establishment of the pharmacognostic profile of the leaves of *M.Koenigii* will assist in standardization which can guarantee quality, purity and identification of sample Different parameters like macroscopy, Microscopy, Phytochemical screening, microbial assay etc were done for standardization.

Significance of different parameters:

Stomatal no. is significant in the evaluation of a leaf drug and affected by factors like age of the plant and size of the leaf. Stomatal index is relatively constant and therefore, of diagnostic significance for a given species and also used for the differentiation of allied or closely related species of the same genus in air dried,as well as fresh conditions. Vein islet and vein termination number is also used as a characteristic for the identification of allied species. Palisade ratio furnishes an important data for leaf drug evaluation and can be successfully applied for the studies of several dicot leaves of medicinal property.

Ash value is helpful in determining the quantity and purity of a crude drug. Loss on drying determines the moisture content and fluorescence study is an essential parameter for first line standardization of crude drug.

Finally, antimicrobial activity can be determined by comparing zone of inhibition of standard solution and test solution (here, methanolic leaf extract).

CONCLUSION:

The pharmacognostic studies of the leaves of *Murraya koenigii* were studied successfully.

REFERENCES

1. Anonymous, *Medical Plants of India* (1987) ICMR, Cambridge orks, New Delhi, 289–295.
2. Chan LW, Cheah EL, Saw CL, Weng W, Heng PW.(2008) Antimicrobial and antioxidant activities of Cortex *Magnoliae Officinalis* and some other medicinal plants commonly used in South-East Asia. *Chin Med* **3**: 15
3. Gupta GL, Nigam SS (1970) Chemical examination of the leaves of *Murraya koenigii*.*Planta Medica* **19**(1): 83–86
4. Iyar D and Devi D (2008). Phytopharmacology of *Murraya koenigii* (L),*Pharmacognosy review*,**2** (3): 180–183
5. Khandelwal KR, (2007) *Practical Pharmacognosy-Techniques and Experiments*.Pune:Nirali Prakashan, 146–170
6. Kokate CK,*Practical Pharmacognosy*,18th Ed, 140–170
7. Math MV, Balasubramaniam P (2005) Physical properties of cold extract of curry leaves (*Murraya koenigii* spreng).*Indian J Physiol Pharmacol*, **49**(4): 491–2.
8. Sasidharan S, Yoga Latha L, Zurani Z, Suryani S, Sangetha S, Shirley L (2007) *Antidiarrheal and antimicrobial activities of the Stachytarpheta jamaicenses leaves Indian Journal of Pharmacology*: **39** (5): 245–248
9. Shanta TR and Shetty JKP (2006) Pharmacognostical study on *Ficus bengalensis* Linn. *Indian J track knowledge*, **5**:388–393
10. *The Ayurvedic Pharmacoepia of India* (1999),**1**: 191–2
11. *The Indian Pharmacoepia* (1996), vol 2, A-100-116

Standardization of 'Dashamularishta': A Polyherbal Formulation

Jain Sanjay*, Srivastava Sweta, Barik Rakesh and Kumar Praveen

Department of Pharmacognosy, Smriti College of Pharmaceutical Education, Madhya Pradesh.

ABSTRACT

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The present work is an attempt to standardize Dashamularishta, a traditional formulation, used in the normalization of physiological processes after child birth. Four marketed preparations and three in-house preparations were used for the study. The various parameters performed included organoleptic characteristics, physicochemical and toxicological parameters. HPTLC was carried out for quantitative analysis of piperine in all the formulations. The results obtained may be considered as tools for assistance to the regulatory authorities, scientific organizations and manufacturers for developing standards.

Keywords: Dashamularishta, standardization, botanical parameters, physico-chemical parameters, toxicological parameters

Editor: Mueen Ahmed KK, Phcog.Net

***For correspondence:** scopeindore@yahoo.com Phone: 0731 – 2802467, Fax: 0731– 2802467

INTRODUCTION

Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principles (1). It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical location. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of Ayurvedic medicines so as to ensure quality control and quality assurance. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.

The present study was aimed at standardizing Dashamularishta, a traditional polyherbal formulation, which is used for the normalization of physiological processes after child birth. It is also used in treating fatigue, removing dead cells from the body, in regeneration of the cells, sedative and tonic (2). It consists of ten main herbs, collectively called as Dashamula class and thirty five other herbs, collectively called as general class of herbs (Tables 1A and B).

MATERIALS AND METHODS

Drug Samples and Method of Preparation (3, 4)

The crude drugs used in preparation of Dashamularishta were collected from local market, Indore and identified in Department of Botany, Government Agriculture College, Indore. Three in house formulations were prepared, with varying concentrations of one of the ingredient, Dhataki, as per the procedure mentioned in Ayurvedic text "Ayurveda Sar Sangrah" and four marketed formulations of different manufacturers were procured. The Dashamula class of herbs were taken in equal proportions (18.30 gm each) and powdered coarsely, then to it the general class herbs (7.32 gm each) were added in powdered form, and prescribed amount (5.5 Ltr) of water was added i.e. 8 times the weight of the herbs taken and kept for boiling until one-fourth (1.5 Ltr) of the decoction was left. Simultaneously jaggery was prepared by taking the prescribed quantity (0.885 gm) of munnaka dissolved in water 4 times (3.5 Ltr) of its weight and was kept on boiling until three-fourth (2.6 Ltr) of its decoction was left. When both decoctions were prepared, they were transferred to

Table I-A:. Dashamula class herbs

S.No	Botanical Name	Common Name
1	*Aegle marmelous*	Bael
2	*Oryxlum indicum*	Sonpataha
3	*Premna mucronata*	Arani
4	*Sterospermum suaveolens*	Patala
5	*Tribulus terristris*	Gokhru
6	*Desmodium gangeticum*	Shalparni
7	*Solanum xanthocarpum*	Choti kateli
8	*Uracaria picta*	Prishparni
9	*Solanum indicum*	Badi kateli
10	*Gmelina arborea*	Gambhir

Table I-B:. General Class of herbs

1	*Plumbago zeylanica*	Chitrak
2	*Inula racemosa*	Puskar moola
3	*Sympiocos racemosa*	Sodhra
4	*Tinospora cordifolia*	Giloy
5	*Acacia catechu*	Khadir
6	*Pterocarpus marsupium*	Vijayasar
7	*Terminalia chebula*	Harad
8	*Terminalia belerica*	Bahera
9	*Sauassurea costus*	Kuth
19	*Cedrus deodara*	Deodar
11	*Embelia ribe*	Vidanga
12	*Piper cubeba*	Kebab chini
13	*Santalum album*	Chandan
14	*Myristica fragrans*	Jaiphal
15	*Asparagus racemosus*	Shatavari
16	*Pueraria tuberose*	Vidarikand
17	*Withania somnifera*	Ashwagandha
18	*Woodfordia fruticosa*	Dhakati
19	*Cinnamomum zeylanicum*	Dalchini
20	*Cinnamomum tamala*	Tejpatra
21	*Eletlaria cardamom*	Badi elachi
22	*Piper longum*	Pipali
23	*Hemidesmus indicus*	Sariva
24	*Mesua ferrea*	Nageskeshwar
25	*Callicarpa marcophyll*	Priyangu
26	*Carum carvi*	Kalazeera
27	*Ipomoea turpethum*	Nisoth
28	*Vitex negundo*	Renuka
29	*Pluchea Lanceolata*	Rasna
30	*Areca catechu*	Supari
31	*Cyperus rotundus*	Nagar motha
32	*Cyperus seariosus*	Jeevak
33	*Pistacia Iintegerrima*	Kakadsingi
34	*Jaggery*	Guda
35	*Vitis vinifera*	Munnaka

three earthen pots in equal amount. To each earthen pot Dhataki was added in different concentration, then other supportive ingredients such as Guda (1400 gm), Pipali, Badi elachi etc were added to it and then it was packed tightly and kept beneath the earth for the period of one month.

The coding of the marketed and in-house formulations was done as follows:

Marketed Preparations

Code	Manufacturer
SD1	Sandu Pharmaceuticals
SD2	Baidyanath Pvt. Ltd.
SD3	Dabur India Ltd.
SD4	Vyas Pharmaceuticals

In-house Preparations

SD5	containing Dhataki + 10% of the prescribed quantity
SD6	containing prescribed amount of Dhataki
SD7	containing Dhataki – 10% of the prescribed quantity

Botanical Parameters (5)

Organoleptic evaluation was carried out to assess the color, odor and taste of the marketed and in-house formulations.

Determination of total solid content(6)

10 ml of the samples were taken in tared dish and evaporated at low temperature until the liquid was removed and then heated until the residue was apparently dried. Thereafter, it was transferred to an oven and dried to constant weight at 105°C.

Determination of specific gravity

The specific gravity was measured using the standard procedure (7) using a pycknometer.

Determination of viscosity (8)

Viscosity was determined with the help of Ostwald's viscometer.

Determination of alcohol content

25ml of the sample was taken in a distillation flask. Then it was diluted with 150 ml of water and little pumice powder was added to it, distillation head and condenser were attached. 90 ml of distillate was collected in a 100 ml volumetric flask and cooled to 25°C. The volume was adjusted to 100 ml. Then the specific gravity of the sample

was measured and then alcohol content was determined as per the table given in I .P. (9)

Determination of sugar content (10)

Sucrose (0.475g) was dissolved in 250ml of distilled water. It was converted into invert sugar, by adding conc. HCl (2ml) to it and boiling gently for 30min. The solution was kept on boiling water-bath for about 2h. and neutralized with sodium carbonate. The neutralized solution was diluted up to 500 ml. 5ml of each sample i.e. SD1, SD2, SD3, SD4, SD5, SD6 and SD7 were taken and to each 25 ml of water was added, followed by 2 ml HCl and boiled for 2 hrs. Then it was filtered and the filtrate was collected and neutralized with sodium bicarbonate and the volume was made up to 250 ml. Fehling's solution was prepared freshly every time, by mixing equal volumes of Fehling's A and B. 10ml of Fehling's solution was taken in porcelain evaporating basin and diluted with equal volume of distilled water. The solution was allowed to boil, and titrated against standard invert sugar solution until the blue color entirely disappeared. Then the solution was allowed to cool till the precipitate of cuprous oxide was settled and the solution was boiled again until the end-point was approached. 5ml of sample was dissolved in water and diluted upto 250 ml, and titrated against 25ml. of the standard Fehling's solution.

Determination of pH (11)

The pH of the all the seven formulation SD1, SD2, SD3, SD4, SD5, SD6 and SD7 was determined with the help of pH meter.

Determination of refractive index (12)

It was determined with the help of Abbes Refractometer.

Determination of acid value (13)

10 ml of sample was taken and dissolved in 50 ml of equal mixture of solvent ether and alcohol. This solution was titrated with 0.1 N NaOH, 1 ml Phenolphthalein was added as indicator and was titrated until the solution remained faintly pink after shaking for 30 sec.

The acid-value of sample was calculated by following formula

$$\text{Acid value} = \frac{n \times 5.61}{w}$$

n = the number of ml of 0.1 N sodium hydroxide required

w = the weight in g of the substance

Quantitative determination of piperine by HPTLC (14)

Chromatographic Condition

- Test plate: HPTLC pre-coated plates (25 TLC Aluminum sheets), silica gel 60 F_{254} (Merck KgaA, 64271 Darmstadt, Germany)
- Format: 10 × 20 cm
- Thickness: 200µm
- Application mode: CAMAG Linomat IV applicator
- Development chamber: CAMAG Twin Trough Chamber
- Densitometric scanning: CAMAG TLC Evaluation software CATS 3.20.
- Detection: Deuterium lamp
- Measurement mode: Absorption/Reflection
- Wavelength : 254 nm
- Solvent System: Acetone: n Hexane 3: 7

Sample and standard (piperine) were prepared as per the standard procedure.

Procedure

The plates were pre-washed with methanol before spotting. Standard and sample solutions were applied to the plates as sharp bands by means of Linomat IV sample applicator. The spots were dried in a current of air. 20 ml of mobile phase was poured into one trough of the Camag twin trough glass chamber and the plate was placed in another trough of the chamber. The whole assembly was left to equilibrate for 30 min. The plates was then developed until the solvent front had traveled a distance of 80 mm above the base of plates. The plate was then removed from the chamber and dried in current of air. Detection and quantification was performed with Camag TLC scanner II at wavelength of 254 nm for evaluation of data.

Quantification of active ingredient

Quantification of active ingredient was done by comparing the peak areas and R_f values of standard piperine with the sample and the % was calculated accordingly.

Microbial contamination (15)

The samples were tested for the presence of microorganisms like *E.coli, Salmonella, Pseudomonas aeruginosa and Staphylococcus aureus*

Heavy metal analysis (16)

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid was added and boiled for 10 minutes

The mixture was cooled down and volume made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, copper, arsenic and mercury.

RESULTS AND DISCUSSION

All the formulations of Dashamularishta were evaluated as per WHO guidelines. Botanical parameters revealed that the formulations were red to reddish brown in color, with pleasant odor and sour taste (table II). The values for percentage of total solid content, specific gravity, viscosity, refractive index, acid value, alcohol content, sugar content and pH in all formulations of Dashamularishta are presented in table III. Alcohol content in the in-house formulation having prescribed amount of Dhataki was found to be 11.0 %. Formulation containing more than prescribed amount of Dhataki contained 22.0 % of alcohol and formulation containing less amount of Dhataki showed no alcohol production. Dhataki is the component responsible for causing fermentation and generation of alcohol in any arishtha preparation (15). The concentrations of Dhataki were varied in the three in-house formulations to observe its effect in generation of alcohol. The results obtained revealed that the amount prescribed in Ayurveda Sar Sangrah is optimum for generation of alcohol. In HPTLC study, the R_f value of standard Piperine was found to be 0.37 where as R_f values of piperine and its quantity in all the formulations were found to be in the range of 0.36–0.38 and 0.11–0.12% respectively (Table IV). The peaks obtained for the formulations matched with that of the standard piperine thereby confirming the presence of piperine in all the samples of Dashamularishta (fig. 1–8).

Various microorganisms like *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* contaminate herbal drugs and cause serious health hazards (16). For detection of such microorganisms, colonies obtained on specific media were subjected to suitable microbial tests along with pure strains to detect their presence or absence. The results obtained (Table V) revealed the absence of these microorganisms thereby confirming the non toxic nature of Dashamularishta. Heavy metals may be present in crude drugs through atmospheric pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations. However, heavy metals have been associated with various adverse effects (17) including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs (18). Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals (Table VI), thereby further confirming the non toxic nature of the preparation. Hence, Dashamularishta is a safe polyherbal formulation and is free from any toxic materials,

Table II: Botanical evaluation of marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Color	Dark brown	Dark reddish brown	Dark reddish brown	Dark brown	Dark brown	Dark brown	Dark brown
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Sour	Sour	Sour	Sour	Sour	Sour	Sour

Table III: Physico-chemical evaluation marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Total solid content	11.42 ± 0.03	16.61 ± 0.02	11.96 ± 0.01	11.78 ± 0.01	12.15 ± 0.00	12.15 ± 0.00	12.63 ± 0.02
Specific gravity	1.07 ± 0.01	1.08 ± 0.00	1.04 ± 0.29	1.11 ± 0.001	1.02 ± 0.02	1.07 ±.006	1.01 ± 0.01
Viscosity	2.90 ± 0.02	2.76 ± 0.02	3.87 ± 0.01	4.18 ± 0.00	2.74 ± 0.01	2.80 ± 0.04	2.83 ± 0.03
Refractive index	1.410 ± 0.001	1.424 ± 0.004	1.413 ± 0.00	1.413 ± 0.01	1.443 ± 0.003	1.433 ± 0.02	1.393 ± 0.003
Alcohol content	12 ± 0.00	10.00 ± 0.33	11.00 ± 0.33	11.00 ± 0.00	22.00 ± 0.33	12.00 ± 0.00	0.00
Sugar content	20.91 ± 0.05	23.48 ± 0.14	19.53 ± 0.13	26.30 ± 0.29	19.17 ± 0.08	20.83 ± 0.01	21.40 ± 0.05
Acid value	3.03 ± 0.00	3.12 ± 0.05	2.96 ± 0.17	2.91 ± 0.25	2.94 ± 0.00	2.80 ± 0.07	2.01 ± 0.18
pH	4.16 ± 0.03	3.91 ± 0.03	4.26 ± 0.00	4.18 ± 0.01	4.83 ± 0.09	4.11 ± 0.04	3.73 ± 0.05

Values are means ± SEM of three experiments.

Table IV: Screening for micro-organisms in marketed and in-house formulations of Dashamularishta

Formulation Code → Name of Microbes↓	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Escherichia coli	–	–	–	–	–	–	–
Salmonella typhi	–	–	–	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–

'–' indicates absence.

Table V: Heavy metal analysis of marketed and in-house formulations of Dashamularishta

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
Arsenic	–	–	–	–	–	–	–
Lead	–	–	–	–	–	–	–
Mercury	–	–	–	–	–	–	–
Copper	–	–	–	–	–	–	–

'–' indicates absence.

Table VI: Quantitative estimation of piperine in marketed and in-house formulations of Dashamularishta by HPTLC

Formulation code	SD1	SD2	SD3	SD4	SD5	SD6	SD7
R_f Values	0.36	0.37	0.37	0.37	0.36	0.38	0.39
Amount of Piperine	0.11%	0.11%	0.11%	0.11%	0.12%	0.12%	0.12%

generally associated with polyherbal preparations. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards for the preparation.

ACKNOWLEDGEMENT

The authors sincerely thank the management of Smriti College of Pharmaceutical Education, Indore, for providing the necessary facilities to carry out the study and Choksi Laboratories, Indore, for carrying out the microbial screening and heavy metal analysis of the formulations and Asthagiri herbal foundation, Chennai, for carrying out HPTLC.

REFERENCES

1) Chakravarthy B K, Standardization of Herbal products, Ind. J. Nat. Products, 9, pp 23–26 (1993).
2) W.H.O.: Quality control methods for medicinal plant materials, Geneva, A.I.T.B.S publishers and distributors, Delhi pp 65–67 (2002).
3) Dwivedi K.M. and Dixit R. : Eleventh Constituent Of Dashmula, Sachitra Ayurveda pp 212–213 (2004).
4) Ayurved Sar Sangrah, Shri Baidyanath Ayurveda Bhawan Pvt Ltd., Nagpur 10th Edition, pp 645 (2000).
5) Agarwal S.S.: Herbal drug technology, University Press (India) Pvt Ltd, Hyderabad, pp 625–679(2007).
6) Mukherjee P.K.: Quality Control of Herbal drugs, 1 edition (Business Horizon, New Delhi, 1999) pp 56–58.
7) Yadav J.B.: Density of Liquids, In: Physical Pharmacy (CBS publishers, New Delhi, 2001) pp 158–159.
8) Subramanyam C.V.S.: Physical Properties of Drug Molecules. In: Essentials of Physical Pharmacy, 3rd edition (Vallabh Prakashan, New Delhi, 1997,) pp 156–165.
9) Indian Pharmacopoeia, (Ministry of Health and Family Welfare, Government of India, New Delhi,) 3rd edition, 1985 Vol II, A-113-115.
10) Jain U. K and Dixit V. K.: Investigation for the development of quality control Method for Chayawanprash, Ind. Drugs, 40, pp 333–339 (2003).
11) Mukherjee P.K. Quality Control of Herbal drug, 1 edition, (Business Horizon, New Delhi, 1999) pp 564–568
12) Subramanyam C.V.S.: Physical Properties of Drug Molecules, In: Essentials of Physical Pharmacy, 3rd edition, (Vallabh Prakashan, New Delhi, 1997) pp 164–166.
13) Indian Pharmacopoeia, (Ministry of Health and Family Welfare, Government of India, New Delhi) 3rd edition, 1985 Vol II; A-71.

14) Wagner H and Bladt S.Z.: Plant Drug Analysis, (Springer-Verlang Berlin Heidal Berg, New York 1983,) pp 352–357.
15) W.H.O.: Quality control methods for medicinal plant materials, Geneva, (A.I.T.B.S publishers and distributors, Delhi, 2002) pp 46–51.
16) Das PK, Goswami S, Chinnaiah A, Panda N, Bannerjee S, Sahu NP and Achari B.: Woodfordia fruticosa: Traditional uses and recent findings. J Ethnopharmacol, 110 (2), pp 189–199 (2007).
17) Dwivedi S.K. and Dey S.: Medicinal herbs: a potential source of toxic metal exposure for man and animals in India. Arch Environ Health, 57, pp 229–231(2002)
18) Moore C. and Adler R.: Herbal vitamins: lead toxicity and developmental delay. Pediatrics, 106, 600–602 (2000).
19) Traub S.J., Hoffman R.S., Nelson L.S.: Lead toxicity due to use of an Ayurvedic compound, J Toxicol Clin Toxicol, 40,322–327 (2002).

Studies of the antifungal and antiviral activity of methanolic extract of leaves of *Grewia asiatica.*

Kumari Sangita[1], Mazumder Avijit[2], Pahwa Shilpa*, Jaju Shivkanya[1]

1. N.I.E.T, Institute of Pharmaceutical Technology, 19, Knowledge Park-II, Greater Noida-201306
2. Dept.of Pharmaceutical Sciences, B.I.T., Mesra, Ranchi

ABSTRACT

The plant *Grewia asiatica* linn. has potential antibacterial activity against a wide range of microorganisms. We have studied the antifungal and antiviral activity of the methanolic extract of leaves of *Grewia asiatica*. The solvent used for the extraction was Methanol. It was found from the MIC studies that the sensitivity pattern of the organism for the extract was found to be decreased in the order: *Candida albicans, Aspergillus thiogenitalis, Penicillium notatum, Penicillium citrinum* and *Aspergillus niger*. So, it was found that it was active maximum against *Candida albicans*. However, *Aspergillus niger* was totally resistant against the extract. The examination of antiviral potency of the extract showed the maximum inhibitory property at a concentration of 1000µg/ml against Urdbean leaf crinkle virus.

Keywords: *Grewia asiatica;* Urdbean plant, Urdbean leaf crinkle virus, MIC, Inoculum, Carborandum powder.

Editor: Mueen Ahmed KK, Phcog.Net

* Sangi_mph04@yahoo.co.in, avijitmazum@yahoo.com, shilpapahwa@yahoo.co.in, shivani_jaju@rediffmail.com, Email id- shilpapahwa@yahoo.co.in

***For correspondence:** Email id- shilpapahwa@yahoo.co.in

INTRODUCTION

The plant *Grewia asiatica* is commonly known as Phalsa or Dhamin belonging to family Tilieaceae. The traditional use of leaves of the plant is application on the pustular eruptions by the tribals of Jharkhand. The ether extract of leaf was found to have antibacterial action against *Staphylococcus aureus* & *Escherichia coli*. The methanolic extract of the leaves possessed antibacterial activity comparable with Ciprofloxocin. So we have conducted the test for antifungal and antiviral properties of the methanolic extract of leaves of *G.asiatica*.

MATERIALS & METHODS

Plant source – The leaves of Dhamin (*Grewia asiatica* linn) were collected from the herbal garden of B.I.T. campus and authenticated by Dr.S.Jha, Dept.of Pharmacognosy, B.I.T., Mesra, Ranchi.

Apparatus & Chemicals – Methanol, Dimethyl Sulfoxide, Griseofulvin, Sabouraud's dextrose agar media, Carborandrum powder.

Test plant for antiviral studies – Urdbean plant

**The fungal strains used were *Candida ablicans* ATCC 10231, *Aspergillus niger* ATCC 6275, *Aspergillus thiogenitalis* MTCC 804, *Penicillium notatum* ATCC11625, *Penicillium funiculosum* NCTC 287 and *Penicillium citriunum* MTCC1256. They are preserved in SDA slants in refrigerator at 4°C.

Preparation of the extract

The methanol was taken for extraction of crude drug from leaves of *G.asiatica* in the soxhlet apparatus. The percentage yield of extract was 8.8%. The stock solution of extract (10mg/ml) and the standard (10mg/ml) were prepared by dissolving in dimethyl sulfoxide (DMSO) & distilled water respectively.

Experimental method

The fungal strains used for the study were *Candida albicans, Aspergillus niger, Aspergillus thiogenitalis, Penicillium notatum, Penicillium funiculosum*, and *Penicillium citrinum*. From the stock solutions (10mg/ml) two sets of four

dilutions (1000, 1200, 1500 and 2000µg/ml) each of leaf extract and griseofulvin were prepared. The Sabouraud's dextrose agar plates were flooded with the liquid culture of fungal strains and dried at 25°C for 30minutes.The sterile whatman filter paper discs (4mm diameter) were soaked in four different dilutions of crude extract and placed in appropriate position of the plates marked as quadrant. Then, the plates were incubated in incubator at 25°C for 24 hrs and diameter of zone of inhibition were measured in mm.

In antiviral studies, it was found that the disease caused by the Urd bean leaf crinkle virus (ULCV) is one of the serious and destructive problems in urd bean (black gram) plants. In the antiviral studies, the stock solution (10mg/ml) of *G. asiatica*, four different concentrations 500, 1000, 1500 and 2000µg/ml were prepared using the solvent DMSO and water. Primary leaves of seedlings were sprayed with the test extract and distilled water (control). The leaf lamina to be inoculated was earlier dusted with the carborandum powder to create minute wounds for virus infection. The inoculations were made by rubbing with a piece of absorbent cotton soaked in the sap inoculum.

RESULTS & DISCUSSION

Determinations of MIC of the methanolic extract of leaves of *Grewia asiatica* (linn) against different fungal strains are shown in Table 1 and determination of diameter of Zone of inhibition (in mm) produced by the methanolic extract of the leaves and its comparison with Griseofulvin against some fungal strains are shown in Table 2.

The MIC values obtained from the methanolic extract of the leaf of *G.asiatica* (linn.) against various tested

Table 1: Determination of MIC of the methanolic extract of leaves of *Grewia asiatica (linn)* against different fungal strains

	Conc.of extract in µg/ ml in SDA media							
Name of Fungi	**0***	**200**	**400**	**800**	**1000**	**1200**	**1500**	**2000**
1. *Candida ablicans* ATCC 10231	+	+	–	–	–	–	–	–
2. *Aspergillus niger* ATCC 6275	+	+	+	+	+	+	+	+
3. *Penicillium notatum* ATCC 11625	+	+	+	+	–	–	–	–
4. *Penicillum citrinum* MTCC 1256	+	+	+	+	+	+	+	–
5. *P. funiculosum* NCTC 287	+	+	+	+	+	+	–	–
6. *Aspergillus thiogentalis* MTCC 804	+	+	+, –	–	–	–	–	–

0* control plate containing no extract; + growth
+, – inhibited; – no growth

Table 2: Determination of diameter of Zone of inhibition (in mm) produced by the methanolic extract of the leaves and its comparison with Griseofulvin against some fungal strains

Name of Fungi	**Extract(µg/ ml)**				**Griseofulvin (µg/ ml)**			
	1000	**1200**	**1500**	**2000**	**1000**	**1200**	**1500**	**2000**
Candida albicans ATCC 10231	8.0	8.5	10.0	12.0	9.0	11.0	12.0	14.0
Aspergillus niger ATCC 6275	4.0	4.0	5.0	7.0	7.5	8.5	10.0	11.5
Penicillium notatum ATCC 11625	10.0	11.0	12.0	14.0	7.0	8.0	10.0	12.0
Penicillium citrinum MTCC 1256	7.0	7.5	9.0	10.0	8.5	9.5	11.0	13.0
Penicillium funiculosum NCTC 287	6.0	6.5	7.5	9.0	8.5	10.0	12.0	14.0
Aspergillus thiogentalis MTCC 804	5.0	5.5	6.0	7.5	8.5	10.5	12.0	13.5

Table 3: Study of inhibition of ULCV infection by methanolic extract of leaves of Grewia asiatica

Treatment concentration (µg/ ml)	No.of plants infected out of 25*						percentage infection	percent inhibition over control
	R1	R2	R3	R4	total	mean		
T1-(500)	18 (25.10)	13 (21.13)	14 (21.97)	13 (21.13)	58 (89.33)	14.50 (22.33)	58%	35.55%
T2-(1000)	9.0 (17.46)	9.0 (17.46)	6 (14.18)	10 (18.44)	34 (67.54)	8.50 (16.80)	34%	62%
T3-(1500)	12 (20.27)	7.0 (15.34)	10 (18.49)	9.0 (17.46)	38 (71.51)	9.5 (17.87)	38%	57.77%
T4-(2000)	11 (19.37)	9.0 (17.46)	7.0 (15.34)	13 (21.13)	40 (73.30)	10 (18.32)	40%	55.55%
T5-control (distilled water)	22 (27.97)	20 (26.58)	23 (28.66)	25 (30.0)	90 (113.19)	22.50 (28.29)	90%	–
Total	72.0 (110.17)	58 (97.95)	60 (98.59)	70 (108.16)	260 (414.8)			

*fig within parenthesis are arc sine angular transformed values

fungal strains showed that the extract is highly active against *Candida albicans*. However, *Aspergillus niger* is resistant against the extract. The sensitivity pattern of the organisms for extract was found to be decreased in the following order: *Candida albicans, Aspergillus thiogenitalis, Penicillium notatum, Penicillium funiculosum, Penicillium citrinum and Aspergillus niger* as shown in Table 1 and 2. The observation suggested that the antifungal principles in the extract have a broad spectrum of activity, which is quite comparable with that of griseofulvin.

In case of antiviral studies, it was found that the test plants previously sprayed with 500, 1000, 1500 and 2000µg/ml concentration of *G.asiatica* extract were recorded as 58, 34, 38 and 48 percent infection of ULCV respectively. The maximum ULCV infection was recorded 90% in control. It was found the maximum inhibitory property at 1000µg/ml and fairly good activity at concentration of 1500 and 2000µg/ml against ULCV.

Study of inhibition of ULCV infection by methanolic extract of leaves of *Grewia asiatica* is shown in **Table 3**

ACKNOWLEDGMENT

The authors wish to thanks Dr. S.M.Prasad, head Dept. of Plant Pathology, Bisra Agriculture University, Kanke, Ranchi for providing facilities for antiviral studies.

REFERENCES

1. Babbar O.P., Joshi, M.N. and Chowdhary B.L. (1983) *Ind. J.Exp. Biol.*, 637–638.
2. Bashir, M., Mughal. S.M. and Malik B.A.(1991) *Pakistan J.of Bot.*,**23**,140–142.
3. Basu B.D. (1975) *Indian Medicinal Plants*, part-I(155–158).
4. Kritikar, K.R. and Basu, B.D. (1975) *Indian Medicinal Plants*, 2nd edition, Vol-**I**, 386–395.
5. Mazumder, R., Mendiratta, T., Mazumder,A., and Mandal,S.C.,(2001) *Ancient Science of Life*, Vol.**XX**, July-October,92–96.

Comparative thin layer chromatographic investigations on sources of Shankhpushpi

Sethiya Neeraj K., Nahata Alok, Dixit Vinod K. *

Department of Pharmaceutical Sciences, Dr. Hari Singh Gour Vishwavidyalaya, Sagar (MP) 470003. INDIA

ABSTRACT

The aim of the present study was to investigate the different plant sources of Shankhpushpi viz., *Evolvulus alsinoides* Linn. (EA), *Convulvulus pluricaulis* Choisy. (CP), *Canscora decussata* Schult. (CD) and *Clitorea ternatea* Linn. (CT) for their identification utilizing the technique of thin layer chromatography (TLC). Shankhpushpi is vigorously advertised for its effects on learning and memory in print and electronic media in India. Various phytoconstituents have been reported in these herbs namely, p-coumaryl derivatives, xanthones, anthocyanins and flavonones. We explored the two phytochemical markers, namely scopoletin and mangiferin for their presence or absence in all the species by performing co-TLC of the ethanolic extracts with the markers. These chromatographic studies led us to conclude that scopoletin is present as a major constituent in all whereas mangiferin is present in CD only.

Keywords: *Shankhpushpi; Evolvulus alsinoides; Convulvulus pluricaulis; Canscora decussata; Clitorea ternatea;* Thin Layer Chromatography; Scopoletin; Mangiferin

Editor: Mueen Ahmed KK, Phcog.Net

***Corresponding Address:** Prof Vinod K. Dixit Department of Pharmaceutical Sciences Dr. Hari Singh Gour Vishwavidyalaya Sagar (MP) 470003. INDIA Tel: +917582264582 Fax: +917582264163 Email: dixitvk2011@rediffmail.com

INTRODUCTION

An ethnopharmacological approach has provided leads to identifying potential new drugs from plant sources, including those for cognitive disorders (1). Shankhpushpi is the name known for *Clitorea ternatea* Linn. (CT) (Papilionaceae), *Evolvulus alsinoides* Linn. (EA) (Convulvulaceae), *Convulvulus pluricaulis* Choisy. (CP) (Convulvulaceae) and *Canscora decussata* Schult. (CD) (Gentianaceae) in indigeneous medicine (2). The monograph in Ayurvedic pharmacopoeia of India describes shankhpushpi as whole plant of *Convulvulus pluricaulis* Choisy. But write in its note that *Clitorea ternatea* and *Evolvulus alsinoides* are used as shankhpushpi in certain parts of India (3). In eastern parts of India, use of *Canscora decussata* for Shankhpushpi has been noted. To minimize confusion and to add scientific validity to herbal formulations, it is necessary that like modern drugs, herbal drugs should also be analyzed and proper quality control techniques be developed to verify the quality and quantity of the herbs added in the formulation. Scopoletin is a major chemical constituent of *Convulvulus pluricaulis* Choisy. (syn: *Convulvulus microphyllus* Sieb ex Spreng) and its TLC densitometric estimation has been given in Quality Standards of Indian Medicinal Plants (5). During the course of our investigations we found presence of scopoletin in *Evolvulus alsinoides* (10). Xanthones have been isolated as main constituents of *C. decussata* (6). Various anthocyanins have been reported in flowers of *Clitorea ternatea* (7). Appreciable work has been done on all herbs for their chemistry. Chromatographic technique is a simple measurement to check the phytoflora of herbs in different bands. The match of R_f with exact postion is a major step for preliminary identification with known marker. As shankhpushpi is a controversial drug of herbal market, variation in biological source is obvious in marketed preparations as different ayurvedic formulations are sold by the common name of shankhpushpi. It was, therefore, considered worthwhile to develop a simple thin layer chromatographic analytical method for the identification of herbs present in a sample.

MATERIALS AND METHODS

CD was collected from the outskirts of Raipur (Chattisgarh. India) in January 2007. The plant was identified by Dr S.C. Agrawal (Department of Botany, CDRI, Lucknow). Other three viz. CP, EA and CT were collected from Bhapel village near Sagar, India. All were authenticated in the Department of Botany, Dr. Hari Singh Gour Vishwavidyalaya, Sagar.

All herbs were shade dried at room temperature and ground into a coarse powder. 120 gm of powdered drug was first defatted with petroleum ether. Then marc was subjected to ethanolic extraction in soxhlet extractor (yields CD: 4.47% w/w; CT: 6.45%.w/w; EA: 11.32% w/w and CP: 9.98%w/w). Extraction was performed according to the method followed in our previous studies (8).

Approximately 10 mg of ethanolic extract was dissolved in ethanol. Scopoletin and mangiferin were dissolved in methanol. Precoated and preactivated TLC plates (E Merck) were used for the analysis.

After screening of solvent systems for achieving the best separation of components, following solvent systems were found to be the best for all the drugs:

Cholroform: Methanol: Toluene (8:1:1) (9)

Butanol: Acetic acid: Water (4:1:2)

The detecting reagents used were

(1) UV at 366 nm

(2) Anisaldehyde- Sulphuric acid reagent

(3) 1% Ferric chloride solution

RESULTS

The presence of Scopoletin in EA, CD, CT and CP was detected as a blue fluorescence spot when exposed to UV at 366 nm (Fig 1). Mangiferin is a xanthone, which showed its presence in Butanol: Acetic acid: Water (4:1:2) after spraying with 1% ferric chloride reagent to give apricot yellow green spot (Fig 2).

DISCUSSION

Thus it can be concluded that scopoletin is present in all the four species whereas mangiferin is present in CD only. The source of shankhpushpi as CD may be confirmed by the presence or absence of mangiferin in the sample.

ACKNOWLEDGEMENTS

The authors would like to express sincere thanks to Laila Impex Research Center, Vijaywada (A.P.), India and

Fig. 1: Comparatives TLC of ethanolic extracts of CD, CT, EA and CP with scopoletin (S) (R_f – 0.81 in UV 366 nm.)

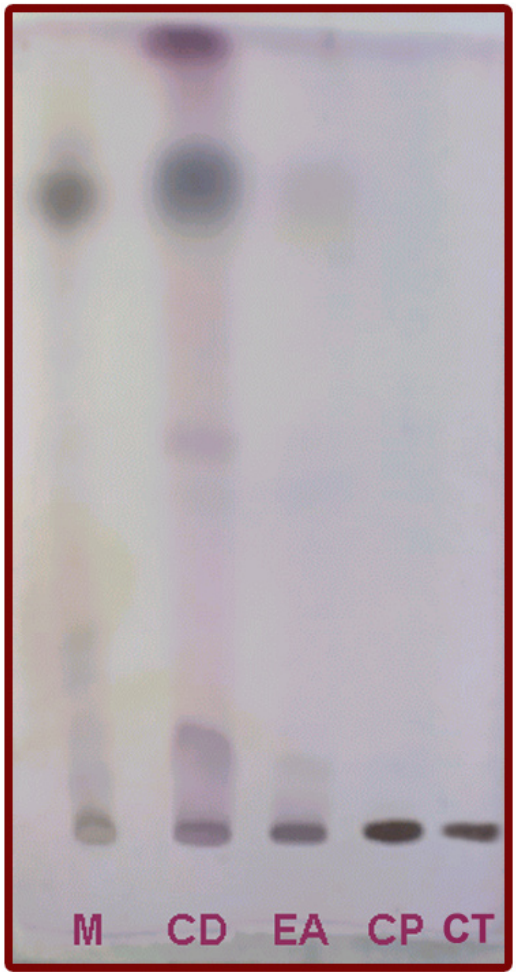


Fig. 2: Comparatives TLC of ethanolic extracts of CD, CT, EA and CP with mangiferin (M) (Rf: 0.72 with 1% $FeCl_3$ sol.)

Natural remedies Pvt Ltd Bangalore, India for providing gift sample of Scopoletin and Mangiferin respectively. This work was supported by UGC.

REFERENCES

1. Jayne M., Howes R., Houghton J. P.. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmcol Biochem Behav 75: 513 (2003).
2. Aulakh G.S., Narayana S., Mahadevan G. "Phytochemistry and Pharmacology of Shankhpushpi four Varieties. Ancient Science of Life 7(3&4):149 (1988).
3. Anonymous. The Ayurvedic Pharmacopoeia of India. 1st Edition, Vol II (Government of India, Ministry of Health and Family Welfare, New Delhi, 1999) 147–149.
4. Chopra R.N., Nayar S.L., Chopra I.C. Glossary of Indian medicinal plants. (Council of Scientific and Industrial Research, New Delhi, 1956) 49.
5. Gupta A.K., Tandon N., Sharma M. Quality standards of Indian Medicinal Plants. Vol 2 (Indian Council of Medical Research, New Delhi, 2005) 70.
6. Ghosal S., Chaudhary R.K., Nath A. Chemical constituents of gentianaceae IV New Xanthone of *Canscora decussata*. J Pharm Sci 62 (1):137 (1973).
7. Terahara N., Toki K., Saito N., Honda T., Matsui T., Osajima Y. Eight New Anthocyanins, Ternatins C1-C5 and D3 and Preternatins A3 and C4 from young *Clitorea ternatea* Flowers. J Nat Prod 61: 1361 (1998).
8. Nahata A., Patil U.K., Dixit V.K. Anxiolytic activity of *Evolvulus alsinoides* and *Convulvulus pluricaulis* in rodents. Pharmaceut Biol 47(5): 444–451 (2009).
9. Nahata A., Patil U.K., Dixit V.K. Effect of *Convulvulus pluricaulis* Choisy. on learning behaviour and memory enhancement activity in rodents. Nat Prod Res 22(16): 1472–82 (2008).
10. Nahata A., Patil U.K., Dixit V.K. Effect of *Evolvulus alsinoides* Linn. on learning behaviour and memory enhancing activity in rodents. Phytother Res, Published Online, DOI: 10.1002/ptr.2932 (2009).

Pharmacognostical Evaluation of *Argyreia speciosa* (Burm.f.) Bojer.

Sandeep Ahlawat*, Karnail Dalal, Arjun Patra

College of Pharmacy, IFTM, Moradabad- 244001, U.P, India

ABSTRACT

The Present study deals with the detailed pharmacognostical evaluation of *Argyreia speciosa* (Burm.f.) Bojer. (Convolvulaceae). Morphoanatomy of the root part have been studied with the aim to aid pharmacognostic and taxonomic species identification. The physicochemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *A. speciosa* and can possibly help to differentiate the drug from its other species.

Keywords: Convolvulaceae, Pharmacognosy, *Argyreia speciosa* (Burm.f.) Bojer.

Editor: Mueen Ahmed KK, Phcog.Net

***Author for Correspondence:** ahlawat1984@yahoo.com

INTRODUCTION

Argyreia speciosa (Convolvulaceae), commonly known as Vryddhadaru in Sanskrit is a woody climber and has been used as a 'rasayana' drug in the traditional Ayurvedic system of medicine. The roots of this plant have been regarded as alterative and tonic, and are said to be useful in rheumatism and diseases of the nervous system (1). It is found throughout India, up to an altitude of 300m, viz., Assam, Bengal, Puri district of Orissa, Dehra Dun, cultivated in Rajasthan, Konkan, Deccan, Mysore. Traditionally, the root is useful in anorexia, Loss of appetite, dyspepsia, flatulence, colic, ascites, haemorrhoids, hemiplegia, nervous weakness, neuralgic pains, cerebral disorders, synovitis and general debility (2). The present investigation includes morphological, anatomical evaluation, determination of physicochemical constants and preliminary phytochemical screening of the hydroalcoholic (1:1) extract and its acetone, methanol and chloroform fraction, ethanol, water, petroleum ether and chloroform extracts and TLC fingerprinting of different extracts of *Argyreia speciosa* were also carried out.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh plant/plant parts were collected randomly from Gujarat region, India and authenticated through Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India (Specimen no. PSN492) and a voucher Specimen has been preserved for further reference. The roots were washed under running tap water; air dried under shade, coarsely powdered and kept in airtight container for further use.

Macroscopic and microscopic analysis

The macroscopy and of the root were studied according to the method of Brain and turner (3). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen (4). The micropowder analysis was done according to the method of Brain and Turner (5) and Kokate (6).

Physicochemical analysis

Physicochemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopoeia (7). Fluorescence analysis was carried out according to the method of Chase and Pratt (8), Kokoski *et al.*(9) and Evans (10).

Preliminary phytochemical screening

Preliminary phytochemical screening for organic and inorganic elements was carried out by using

standard procedures described by Harborne (11) and Khandelwal (12).

Thin layer chromatography

Thin layer chromatography was performed using standard method of Burger, (13) and Janchen *et al*., (14).

RESULTS AND DISCUSSION:

Macroscopic characters

Thin pieces of roots of *Argyreia speciosa* are usually of 2.4mm in diameter with somewhat smooth brownish exterior, while thicker pieces are 5.25 mm or even more in diameter, tough and woody, light brown in colour, rough, longitudinally striated, lenticellate and with circular root scars; fracture fibrous; rootlets and branches, thin and somewhat fibrous; odour, nil; taste, pungent, bitter and astringent.

Microscopic characters

Transverse section

Structurally the woody climber's root consists of epidermis, cortex, secondary phloem, phloem fibres, medullary rays, cambium, secondary xylem, parenchymatous cells and pith. Epidermis which is a single layer consists of small tangentially elongated rectangular cells with brownish, thick-outer walls. Cortex section comprises of 3 to 6 layers of cortical cells (fig. 1a). These are thin walled tangentially elongated cells, some containing starch grains and crystals of calcium oxalate. Secondary phloem is a wide zone consisting of phloem parenchyma, traversed by narrow medullary rays. Secondary xylem is composed of large xylem vessels. Vessels are drum shaped, short in length but large in diameter, having bordered pits on the walls and large perforation rims (fig. 1b). Medullary rays are narrow towards the pith and become wider towards the cortex region (Fig. 1c). Towards pith, it consists of 2 to 4

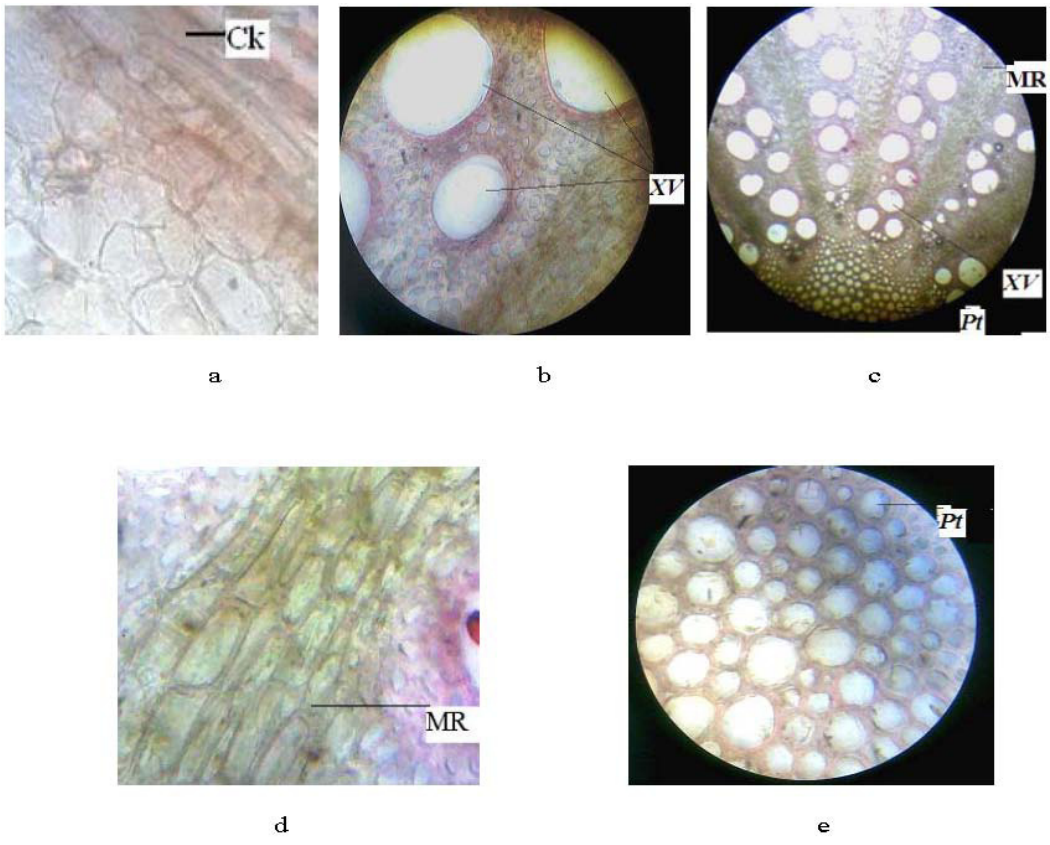


Figure 1. *"Microscopy of root of A. speciosa"*
a; Transverse section consists of Cortex (100x)
b; Transverse section contains xylem vessel (100x)
c; Transverse section consists of pith, xylem vessel and medullary rays (100x)
d; Transverse section consists of medullary rays (100x)
e; Transverse section consists of Pith (100x)
Abbreviations: Ck-Cortex, MR-Medullary rays, Pt-Pith, XV-Primary xylem.

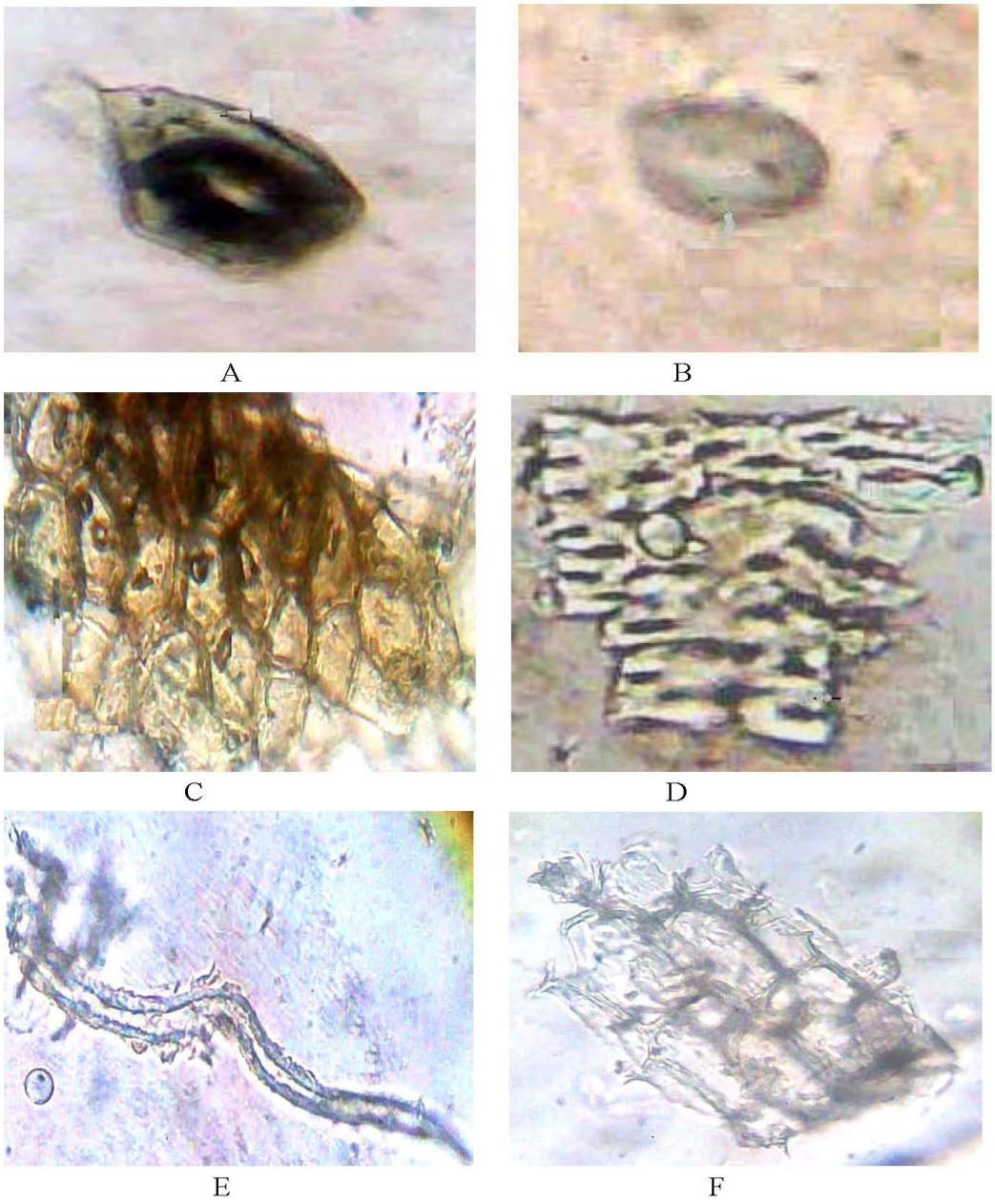


Figure 2. *"Powder microscopy of A. speciosa"*
A: Prismatic Calcium Oxalate, B: Circular Starch Grain, C: Cork Cells, D: Reticulate Vessel, E: Fibre having pointed ends, F: Xylem Vessel.

layers of cells and towards cortex region; it consists of 4 to 6 layers of cells (Fig. 1d). Cambium consists of 2 to 3 rows of small irregular thin-walled cells. Pith consists of few round to oval thin walled parenchymatous cells (fig. 1e).

Powder characteristic

The presence of prismatic crystals of calcium oxalate, starch grain, Cork cell, reticulate vessel, fibres and xylem vessel were observed (Fig. 2).

Preliminary phytochemical screening

Preliminary phytochemical screening for organic and inorganic elements was carried out by using standard procedures. The result of organic elements revealed the presence of alkaloids, carbohydrates, saponin, amino acid, steroids, flavonoids and tannins (Table 1) and the result of inorganic elements shows the presence of iron, phosphate, sulphate, and chloride (Table 2).

"Table 1: Preliminary phytochemical screening of different extracts of A. speciosa root (organic elements)"

Phytoconstituents	Hydro-alcoholic extract	Chloroform extract	Acetone extract	Alcoholic extract	Pet. Ether extract	Water extract	Acetone Fraction	Chloroform Fraction	Methanol Fraction
Alkaloids									
Mayer's test	+	–	–	+	–	+	+	–	–
Dragendroff's Test	+	–	–	+	–	–	+	–	–
Wagner's test	+	–	–	+	–	–	–	–	–
Hager's test	–	–	–	–	–	–	–	–	–
Carbohydrates	+	+	–	+	–	+	–	–	+
Amino acid	+	–	–	+	–	+	–	–	+
Protein									
Biuret test	–	–	–	–	–	–	–	–	–
Xanthoprotein test	–	–	–	–	–	–	–	–	–
Million's test	–	–	–	–	–	–	–	–	–
Saponin	–	–	–	–	–	+	–	–	–
Tannin									
Ferric chloride test	+	–	–	+	–	–	–	+	–
Lead acetate test	+	–	–	+	–	–	–	+	–
Steroid									
Liebermann-Burchard test	+	+	–	+	–	–	–	+	–
Salkowski's test	+	+	–	+	–	–	–	+	–
Flavonoids	+	–	–	+	–	–	–	–	+

(+) Sign indicates presence, (–) Sign indicates absence.

"Table 2: Inorganic constituents of root powder of A. speciosa"

Elements	Results
Calcium	–
Magnesium	–
Sodium	–
Potassium	–
Iron	+
Sulphate	+
Phosphate	+
Chloride	+
Carbonate	–
Nitrates	–

(–) Not present, (+) present

"Table 3: Physicochemical parameters of A. speciosa"

Parameters	Values (%w/w)
Alcohol soluble extractive	11.12%
Water soluble extractive	22%
Chloroform soluble extractive	0.72%
Petroleum ether soluble extractive	0.4%
Acetone soluble extractive	1.44%
Moisture content (LOD)	10.67%
Total ash	4.4%
Acid insoluble ash	0.76%
Water soluble ash	4.03%

"Table 4: TLC of different extracts of root of A. speciosa"

Extracts	Solvent system	Number of spots and their Rf values
Alcoholic Extract	Toluene: Acetone: Methanol (2:1:1)	Three (0.13, 0.58, 0.93)
Chloroform Extract	Toluene: Cyclohexane: Acetic Acid (2:2:2)	Three (0.19, 0.55, 0.82)
Hydroalcoholic Extract	Toluene: Acetone: Methanol (2:1.5:1.5)	Three (0.11, 0.50, 0.64)

"Table 5: Fluorescence analysis of the root powder of Argyreia speciosa"

Treatment		UV light	
	Day light	(254 nm)	(365 nm)
Powder as such	Light Brown	Greenish Brown	Dark Brown
Powder + (50%) HNO_3	Reddish Brown	Green	Black
Powder + 1N HCl	Brown	Green	Brown
Powder + Conc.H_2So_4	Black	Black	Dark Black
Powder + Ammonia	Dark Brown	Light Green	Black
Powder + Iodine	Brown	Green	Brown
Powder + (10%) $BaCl_2$	Creamish Brown	Light Green	Brown
Powder + (40%) NaOH	Brown	Light Green	Dark Brown
Powder + (10%)$AgNO_3$	Light Brown	Light Green	Violet
Powder + (5%) $FeCl_3$	Greenish Brown	Dark Green	Black
Powder + (10%)$MgSo_4$	Light Brown	Light Green	Brown
Powder + (10%)$FeSO_4$	Green	Green	Black
Powder + (5%) $CaCl_2$	Brown	Light Green	Brown
Powder+(40%)$Pb(CH_3COO)_2$	Yellowish Brown	Light Green	Brown
Powder + (5%) $CuSO_4$	Greenish	Green	Black
Powder+Bromine H_2o	Reddish Brown	Green	Black

Physicochemical studies

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out in (Table 3). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble, acetone soluble, chloroform soluble and petroleum ether soluble extractive values have been tabulated in (Table 3). The results of fluorescence analysis of the root powder are presented in (Table 5).

Thin layer chromatography

The TLC of hydroalcoholic, alcoholic and chloroform extracts of *Argyreia specios* root was performed and the solvent systems were developed by running the plate on trial basis in different solvent systems in different ratio. The number of spots and their R_f values has been tabulated in (Table 4).

CONCLUSION

The present study on pharmacognostical evaluation of *A. speciosa* will provide useful information for its identification. Macro, micro and physiochemical standards discussed here can be considered as the identifying parameters to substantiate and authenticate the drug.

ACKNOWLEDGEMENT

The authors sincerely thank I.F.T.M, Moradabad for providing the necessary facilities to carry out the study.

REFERENCES

1. ChadhaY.R.. *The Wealth of India, A Dictionary of Indian Raw Materials & Industrial Products*, Publications and Information Directorate, CSIR, New Delhi, India, **1**: 116 (1976).
2. Sharma P.C., Yelne M.B. and Dennis T.J.. *Database on medicinal Plants used in Ayurveda*, Central Council for Research in Ayurveda and Siddha, Department of ISM&H , Ministry of Health and Family Welfare (Govt. of India), New Delhi, **2**: 550–559 (2005).
3. Brain K.R. and Turner T.D.. *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol. 4–9 (1975a).
4. Johansen D.A.. *Plant Microtechnique*, McGraw Hill, New York. 182(1940).
5. Brain K.R. and Turner T.D.. *The Practical Evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol. 36–45(1975b).
6. Kokate C.K.. *Practical Pharmacognosy*, 1st ed., Vallabh Prakashan, New Delhi. 15–30(1986a).
7. *Indian Pharmacopoeia*, 4th ed., Vol. **II**, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi. A53–A54 (1996).
8. Chase C.R. and Pratt R.J.. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. Am. Pharmacol. Assoc*. **38**: 32 (1949).
9. Kokoski J., Kokoski R. and Slama F.J.. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J. Am. Pharmacol. Assoc.*, **47**: 715 (1958).
10. Evans WC. *Pharmacognosy*, 15th ed. Edinburg London. **475**, 101–105(2002).
11. Harborne J.B.. Methods of extraction and isolation. In: *Phytochemical Methods*, Chapman & Hall, London. 60–66(1998).
12. Khandelwal K.R.. *Practical Pharmacognosy*, Nirali Prakashan. 149–157(2008).
13. Burger K.. *Instrumental Thin Layer Chromatography/Planar Chromatography*, Proceeding of the Instrumental Symposium, Brighton, UK. 33–44 (1989).
14. Janchen D. and Issaq H.J.. *J. Liq. Chromatography*. **11**: 1941–1965 (1988).

Pharmacognostical Profiling of Roots of *Salacia prenoides L.*

Dholwani Kishor kumar*, Saluja Ajay K, Bhatt Shibani, Chauhan Namrata

* Department of Pharmacognosy, A.R College of Pharmacy, Mota bazaar, Vallabh Vidhyanagar, Anand, Gujarat – 380015

ABSTRACT

Salacia prenoides or *S.chinensis* belonging to the family *Hippocrateaceae* is a climbing shrub known as saptarangi in Ayurveda. The roots of this plant is repoted to possess various medicinal uses as antidiabetic, astringent and abortificient. The pharmacognostical study of roots of this plant has not been reported. Therefore, the present investigation was planned to study the morphology, microscopy, physicochemical parameters, phytochemical screening and HPTLC study of roots of *Salacia prenoides*.

Keywords: *Salacia prenoides, Salacia chinensis, saptarangi, microscopy, mangiferin.*

Editor: Mueen Ahmed KK, Phcog.Net

***Author for correspondence:** kishoredholwani@yahoo.co.in

INTRODUCTION

The plant *Salacia prenoides* (synonym: *Salacia chinensis*) commonly known as saptarangi in ayurveda belongs to family *Hippocrateaceae* (1). It is a climbing shrub and its leaves are ovate to lanceolate. Flowers 2–6, clustered together on axillary tubercules, yellowish. Fruit are small, globose, red when ripe, one-seeded. The plant is found throughout India. Roots have been used as an antidiabetic drug in the indigenous system of medicine and clinical tests are said to have substantiated their efficacy. Roots are astringent and abortifacient and are useful in amenorrhoea, dysmenorrhoea and veneral diseases (2, 3). The phytochemical studies reveal dulcitol, mangiferin and gutta, a dimer of luecopelargonidin along with 3 unidentified proanthocyanidins from stems and leaves. New triterpene constituents, foliasalacins A1-A4, B1-B3 and C were found in leaves (4 5). Three new -type triterpenes named salasones A, B and C, a new nonfriedelane-type triterpene, salaquinone A and a new acylated friedelane eudesmane-type sesquiterpene, salasol A were isolated from 80% aqueous methanolic extract of stem (6, 7). Mangiferin is reported in methanolic root extract (8, 9). In spite of the numerous medicinal uses attributed to this plant, pharmacognosy information about this plant has not been published. The present investigation was planned to study the morphology, microscopy, physicochemical parameters, phytochemical screening and HPTLC study of *Salacia prenoides*.

MATERIALS AND METHODS

Plant material

The roots of *Salacia prenoides* were collected from Gujarat-Madhya Pradesh Border area at Kunvaro dungar near Bhilora sanctuary of Dhanpur taluka in Gujarat and authenticated by Department of Bioscience, Sardar Patel University, Vallabh Vidyanagar.

Instruments and chemicals

Compound microscope, microtome, glass slides, cover slips, watch glass and other common glasswares were the basic apparatus and instruments used for the study. Solvents viz. phloroglucinol, glycerine, hydrochloric acid, chloral hydrate and sodium hydroxide were procured from the Pharmacognosy laboratory for Research in A R College of Pharmacy.

Macroscopic and Microscopic analysis

The macroscopy and microscopy of the root were studied according to the method of Brain and Turner. (10, 11) For microscopical studies, cross sections were prepared and stained as per procedure of Johansen. (12, 13) Morphological studies such as shape, size, surface, colour, taste and odour of roots were carried out. Microscopical studies were carried out and transverse sections were taken using microtome. The average thickness of the section was 12–14µ. The sections were stained with toluidine

blue. The section showed pink colour when stained with a mixture of 2 drops of phloroglucinol and one drop of hydrochloric acid kept for 5 minutes. Iodine solution was used for detection of starch. (10, 11).

Physico-chemical analysis

The total ash, water soluble ash, acid insoluble ash, alcohol soluble and water soluble extractive value were performed. Here 5 gms of powder of roots of the plant was taken in a silica crucible whose weight is predetermined and it is kept for incineration according to the official methods prescribed and WHO guidelines (12, 13).

Preliminary phytochemical screening

The dried powder of roots was also subjected to identification tests for detection of various phytoconstituents like alkaloides, glycosides, polyphenols, flavanoides, coumarins, proteins, carbohydrates, gums and mucilage, fixed oil and volatile oil (14).

HPTLC studies–

Qualitative densitometric HPTLC analysis was performed to confirm the presence of mangiferin in methanolic extract of entire plant with authentic sample. The adsorbent used was silica gel 60 F_{254}. The solvent system taken was ethyl acetate : formic acid : glacialacetic acid : water in 100 : 11 : 11 : 27. The quantity applied was 10µl. Solvent was run upto 8cms. The scanning was done at 254 nm. Applicator instrument used is Linomat IV – CAMAG. The densitometer is from Shimadzu.

Figure 1A: Morphology of roots of Salacia prenoides

RESULTS AND DISCUSSION

The root is yellow in colour and has exfoliated bark (Fig. 1A). It has characteristic odour and is bitter in taste. It has rootlets on its surface and its transverse section shows clear annual rings (Fig. 1B). In microscopic studies, the section of root shows a distinguishing characteristic having a wavy cork. It consists of 6–7 layers (Fig. 1E). The cortex is a larger portion consisting of brown matter. It has stellar region having secondary growth and the cells are mainly parenchymatous in nature. Starch is present

Figure 1B: Annual rings in roots of Salacia prenoides.

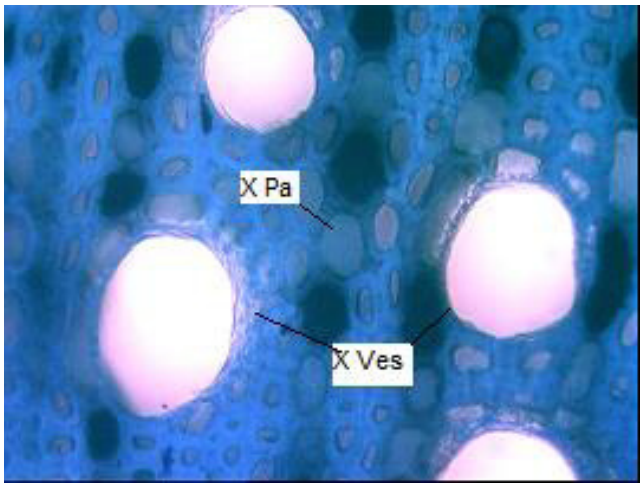


Figure 1C: T.S. of root showing xylem portion. (X Pa-Xylem parenchyma; X Ves- Xylem vessel)

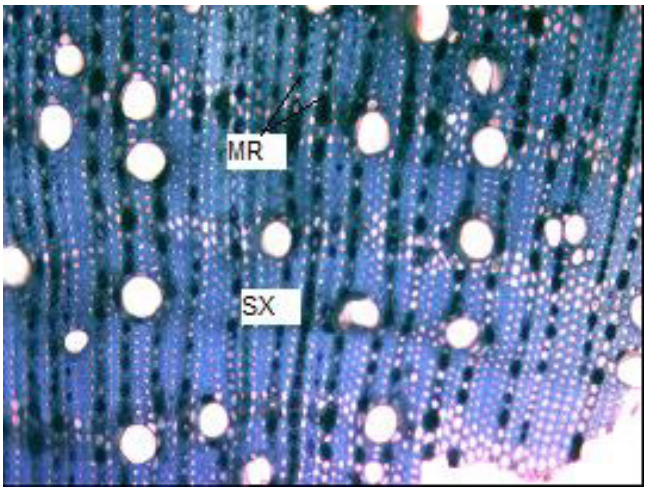


Figure 1D: T.S. of root of secondary growth. (MR- Medullary rays; SX- Secondary xylem)

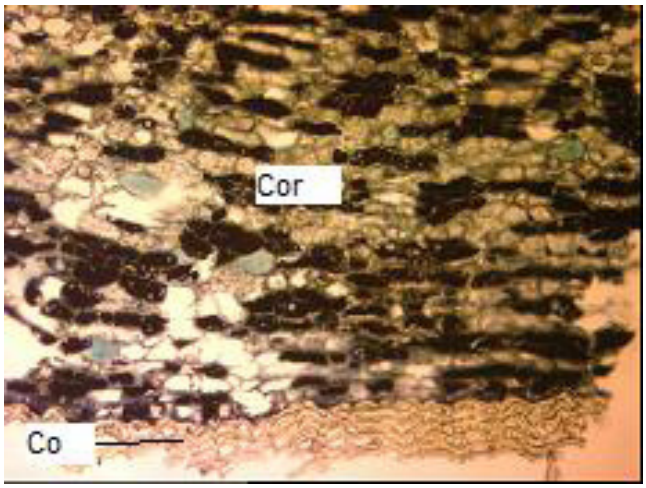


Figure 1E: T.S of root of wood portion. (Co- Wavy cork; Cor- Cortex with brown matter)

Table-2: Ash values of root powder of Salacia prenoides L.

Parameter	Result
Total ash	4.825%w/w
Water-soluble ash	2.75%w/w
Acid-insoluble ash	3.5 %w/w

Table-3: Extractive values of root powder of Salacia prenoides L.

Water-soluble extractive value	3.275%w/w
Alcohol-soluble extractive value	1.8%w/w

Table-1. Preliminary phytochemical screening of the entire plant powder of S. prenoides

Test	Petroleum ether	Toluene	Chloroform	Acetone	Ethanol	Water
Alkaloids	–	–	–	–	+	–
Carbohydrates	–	–	–	–	+	+
Phytosterols	+	+	–	–	+	–
Fixed oil and fats	–	–	–	–	–	–
Saponin	–	–	–	–	–	–
Polyphenols	–	–	–	–	+	+
Coumarins	–	–	–	–	+	+
Flavanoids	–	–	–	–	+	+
Proteins	–	–	–	–	–	–
Gums and mucilage	–	–	–	–	–	+
Volatile oils	–	–	–	–	–	–

+ Denotes the presence of the respective class of compounds

in the cortex region. The vascular bundle consists mainly of secondary xylem and phloem.

Distinct xylem vessels and xylem parenchyma were observed (Fig. 1C). The section also shows uniserriate and few biserriate medullary rays (Fig. 1D). Presence of starch is detected in medullary rays. Pith is absent. Pericycle and endodermis is also found to be absent.

Preliminary photochemical screening - Preliminary phytochemical screening revealed the presence of alkaloids, phytosterols, polyphenols, coumarins, flavonoids and carbohydrates (Table 1).

Physicochemical studies - Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash are carried out (Table 2). Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble

Table-4: Fluorescence analysis of root powder of S. prenoides

Treatment	Day light	UV light(254nm)
Powder as such	Yellow color	Light green
Powder + 1N NaOH(Aq.)	Brown	Dark brown
Powder + 1N NaOH(Alc.)	Yellowish brown	Light yellow
Powder + 1N HCL	Green	Light green
Powder + Iodine	Dark brown	Brown
Powder + Ammonia	Yellow	Greenish yellow
Powder + 5% $FeCl_3$	Dark yellow	Dark brown
Powder + 1N H_2SO_4	Black	No color
Powder + Acetic acid	Light brown	Orange
Powder + 1N HNO_3	Brown	No color

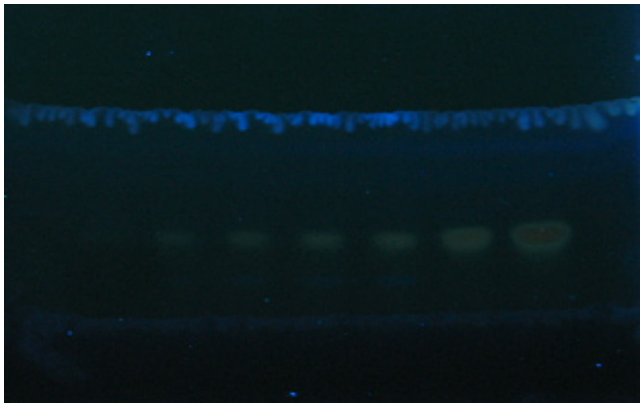


Figure 2A: Fluorescence observed in TLC plate of extract of S. prenoides.

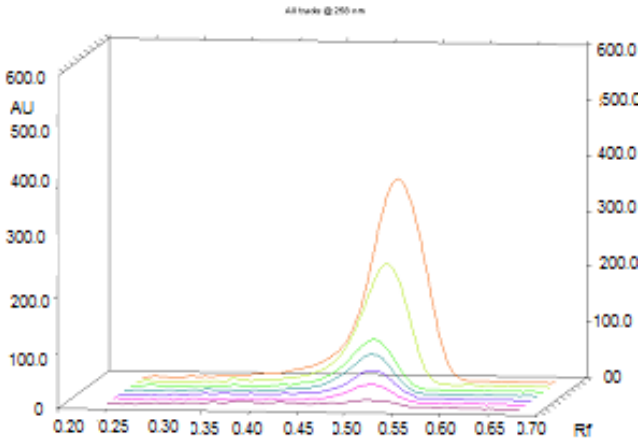


Figure 2C: 3D graph including standard extract.

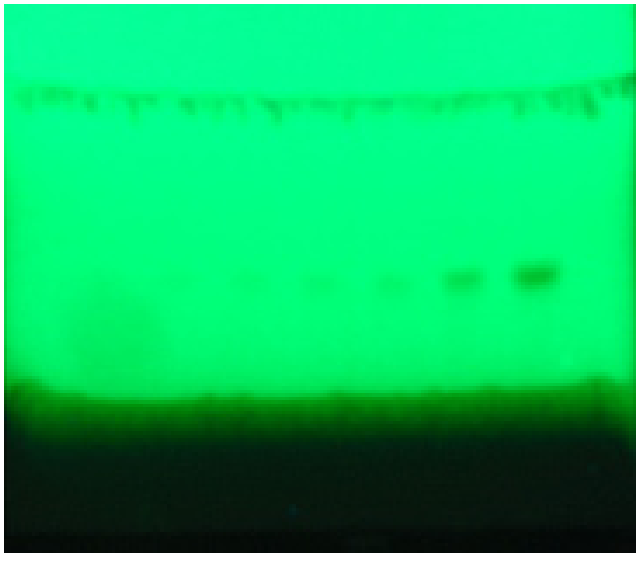


Figure 2B: UV photo of root extract of S. prenoides.

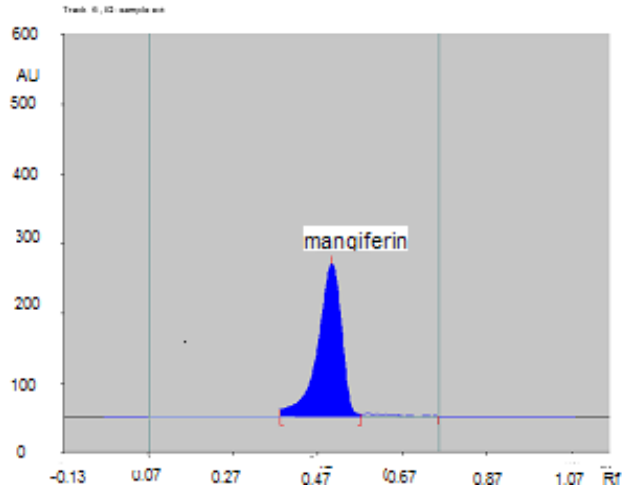


Figure 2D: HPTLC profile of methanolic extract of S. prenoides indicating presence of mangiferin.

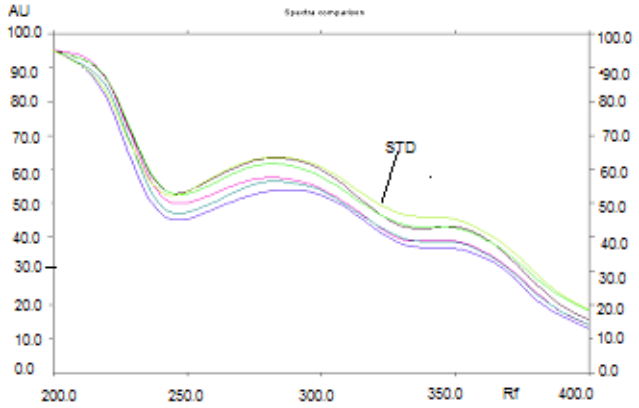


Figure 2E: UV spectra of sample + standard.

values have been tabulated in Table 3. The results of fluorescence analysis of the drug powder are presented in Table 4.

HPTLC studies - A qualitative densitometric HPTLC analysis was performed to confirm the presence of mangiferin in methanolic extract of root powder. Mangiferin content in the powder drug was found to be 0.50%w/w.

CONCLUSION

The present study on pharmacognostical evaluation of roots *Salacia prenoides* will provide useful information for its identification. Macro, micro and physiochemical standards discussed here can be considered as the identifying parameters to substantiate and authenticate the drug.

ACKNOWLEDGEMENT

The authors sincerely thank A.R college of Pharmacy for providing the necessary facilities to carry out the study.

REFERENCES

1. Mehra and Handa. Researchers in pharmacognosy in India, *Bulletin of Punjab University*. **20**:275 (1969).
2. *Encyclopaedia of World Medicinal Plants* 1713.
3. Almeida MR and Almeida SM, *Folklore and utility of vascular plants from Savantwadi*, Alchemie Research Centre. Thane. 27.
4. *Bull. Res*. Inst. Univ. Kerala, Trivandrum.**5**: 29 (1957).
5. Yoshikawa M, Zhang Y, Wang T, Nakamura S and Matsuda H; *Chemical and Pharmaceutical bulletin*. **56**(7): 915-920 (2008).
6. Morikawa T and Yoshikawa M. Structures of new friedelane-type triterpenes and eudesmane type sesquiterpenes and aldose reductase inhibitors from *Salacia chinensis*. *J. Nat. Prod* **66**: 1192-1196 (2003).
7. Subbaraju GV, Kumar KK, Raju BL, Pillai KR, Reddy MC, *J. Nat. Prod*.**54**: 1639-1641 (1991).
8. Sellamuthu Periyar, Muniappan Balu, perumal Sathiya, Kandasamy Murugesan. Antihypertensive effect of Mangiferin in Streptozocin induced diabetic rats, *Journal of Health Science*. **55**(2): 206-214 (2009).
9. Dar Ahsana, Faizi Shaheen and Firdous Sadiqa. Analgesic and Antioxidant activity of Mangiferin and its Derivatives: the Structure Activity Relationship, *Biol. Pharm. Bull*. **28**(4): 596-600 (2005).
10. Brain K.R. and Turner T.D. *The practical evaluation of Phytopharmaceuticals*, Wright-Scientechnica, Bristol. 4-9 (1975a).
11. Khandelwal KR, *Practical Pharmacognosy, Techniques and experiments*, Edn12, Nirali Prakahsan, 9 (2002).
12. Johansen D.A.. *Plant Microtechnique*, McGraw Hill, New York, 182 (1940).
13. Kokate CK, *Practical Pharmacognosy*, Edn4, Vallabh Prakashan, Delhi, 7-14 (2005).
14. Anonymous, *Indian Pharmacopoeia, Vol2, Ministry of Health and Family Welfare*, Govt. of India, Controller of publication, New Delhi.