Pharmacognostic Studies and Standardization of *Cassia Sieberiana* Roots

Sam, GH, Mensah MLK and Nyakoa-Ofori N.

Department of Pharmacognosy, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.

ABSTRACT

Cassia sieberiana D. C. [Caesalpiniaceae] though widely used in traditional medicine as an analgesic in dysmenorrhoea, ulcer, general body pains and in veterinary medicine, there is no report on its standardization. **Objective:** To establish standards for a partial monograph preparation. **Materials and methods:** Standardization followed British Pharmacoepoeia Standardization methods. Plant materials were collected and air dried for five days and then oven dried at 52°C for one hour and milled. A quantity of *C. sieberiana* powdered root was mounted separately in each of 10% iodine, chloral hydrate, phloroglucinol in conc HCl and Sudan III and examined under the microscope to identify the cell types and cell inclusions present. Mounted slides were photographed using digital camera Olympus OIL-8CC. Total ash value, acid insoluble ash, solvent extractives, pH and phytochemical analysis of extracts followed methods as in British Pharmacoepia (BP) 1990. **Results:** Transverse section (TS) of the root bark shows outermost layer of cork, beneath which is a layer of parenchyma cells and a ring of stone cells. The longitudinal section (LS) of the root bark conforms to the same tissue arrangement. Calcium oxalate crystals are pyramidal shaped with base diagonals measuring 40 to 160µ. Starch grains are oval; length ranging from 60 to 180µ and diameter from 40 to120µ. Total ash; 7.43-8.37, Acid insoluble ash; 4.80-5.50% w/w. Ethanol$_{(70\%)}$ extractive; 14.28-14.30% w/w; Chloroform-water extractive; 15.99-19.98%w/w, pH (1% w/v at 28°C); 4.88. Constituents: Anthraquinones, Saponins, Sterols, Steroidal glycosides, Tannins, Triterpenes. **Conclusion:** Established standards can form the basis for monograph compilation

Keywords: Cassia sieberiana, micromorphology, root, standardization.

INTRODUCTION

Plants have been used for various purposes since prehistoric times[1],[2],[3] *Cassia sieberiana* root bark and rootlets are chewed to treat abdominal pains,[4] as diuretic, for tooth ache, skin diseases, as antihelminthic, haemorrhoids, leprosy,[5],[6] and also widely used in veterinary medicine in Burkina Faso.[7] The plant belongs to the family Caesalpiniaceae R.Br[8] and grows in savannah forests, thickets and coastal scrubs.[4] The first report of the chemical constituents of *C. sieberiana* is probably that by Tambora and co workers in Burkina Faso.[7] The analgesic property of the root has also been reported.[5],[9] The root powder is reported to competitively antagonize acetylcholine and histamine in an isolated guinea pig ileum.[10] These findings lend credence to the use of the plant as an analgesic. With the current level of usage of *C. sieberiana* root it will be a great help if the root of the plant used as the drug is standardized. This work therefore aimed at conducting scientific study to standardize *Cassia sieberiana* root.

Standardization is the process of developing and agreeing upon technical specifications associated with a functional product. A document that establishes the technical specifications, criteria, methods, processes, or practices forms the standard.[11] In this work British Pharmacopoeia and other established methods were followed in the standardization studies.

Partial monograph of *Cassia sieberiana*

Definition

Sieberiana root consists of the dried root bark and rootlets of *Cassia sieberiana* D. C. (pseudonym: *Cassia kotschyana* Oliv.; *Senna sieberiana* DC. [Caesalpiniaceae]

Botanical name: *Cassia sieberiana*

Address for correspondence:
E-mail: ghsam.pharm@knust.edu.gh

DOI: 10.5530/pj.2011.21.2

Family: Caesalpiniaceae

Common English name: West African laburnum, drumstick tree.

Vernacular name Akuko bewu, pirto rodom (Twi in Ghana)

Plant material of interest: Dried root bark and rootlets

Macroscopical characteristics The outer surface of mature root bark is brown and inner, buff in colour. The dried bark is curved with convex outer surface

Microscopic characteristics

The transverse section (TS) of the root bark shows outermost layer of cork, a wide cortex made up of a ring of parenchyma cells, a continuous band of stone cells, stone cells in groups and fibres. The phloem shows large sieve tubes interspersed with phloem parenchyma and fibres. The longitudinal section (LS) of the root bark shows the same tissue arrangement. The powdered root bark and rootlets showed cork cells, fibres, stone cells, sieve tubes of phloem, xylem vessels, parenchyma and collenchyma cells.

Cell inclusions: Pyramidal shaped calcium oxalate crystals with base diagonals measuring 40 to 160 μ (average = 60μ)

Starch grains: Oval in shape with the length, *l*, ranging from 60 to 180 μ and diameter, *d*, ranging from 40 to120 μ (average: *l* = 150 μ, d = 50 μ)

Organoleptics: Root powder has a dark brown colour, bitter taste and has a characteristic odour.

General identity test Characteristic macroscopic and microscopic features

Purity test

Total ash	7.43-8.37% w/w (Average: 7.90% w/w)
Acid insoluble ash	4.80-5.50% w/w (Average: 5.15% w/w)
$Ethanol_{(70\%)}$ extractive	14.28-14.30%w/w (Average: 14.38% w/w)
Chloroform-water extractive	15.99-19.98% w/w (Average: 17.99%w/w)
pH (1% w/v at 28°C)	4.88
Constituents	Anthraquinones, Saponins, Sterols, Steroidal glycosides, Tannins, Triterpenes

Actions and uses Anticholinergenic and anti-histaminic. Strongly antispasmodic. Root used as an analgesic and worm expellant

MATERIALS AND METHODS

Plant materials were collected from Mamfe–Akwapem in the Eastern Region of Ghana in August, 2009 and authenticated by Mr. Ofori–Lartey, a horticulturist in charge of the Herbarium at the Centre for Scientific Research into Plant Medicine, Mampong Akwapem, Ghana. A voucher specimen number KNUST/HM1/09/L 027 was deposited in the herbarium of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi. The root bark was cut into small pieces and air dried for five days and then oven dried at 52°C for one hour after which they were milled. The powder was put into a plastic container and stored in the project laboratory in the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, Kumasi till they were needed

Reagents and chemicals

Except where otherwise stated all chemicals and reagents were purchased from Sigma Company, USA. Pre-coated silica gel chromatographic plates were obtained from Merck, Germany.

Micromorphological studies of root bark

Transverse section (TS) of root bark

Fresh root bark was peeled and air-dried for five days to stabilise constituents and soaked in chloroform water overnight to soften. A segment from the bark measuring about 5 mm × 3 mm was cut out and sectioned (free hand) with a razor blade. The sections were mounted in chloral hydrate over night to clear and examined under the light microscope, Olympus-AX7320.

The best sections were mounted in phloroglucinol in conc. HCl. Another set of sections were mounted separately in 10% iodine, Sudan III and sodium picrate to characterise constituents of various tissues in the root bark.

Longitudinal section (LS) of root bark

The LS of the root bark was prepared in the same way as the TS

Cell types in the powdered root

A small quantity of *C. sieberiana* powdered root was mounted separately in each of 10% iodine, chloral hydrate, phloroglucinol in conc HCl and Sudan III and examined under the microscope to identify the cell types and cell inclusions present in the root powder. All specimens examined under the microscope were photographed using digital camera for microscopy, Olympus OIL-8CC

Phisicochemical studies

Physicochemical determinations, total ash, solvent extractives, pH and phytochemical analysis of extracts followed methods as in British Pharmacoepia (BP) 1990[12] and British Herbal Pharmacopoeia[13]

Thin layer chromatography (TLC) profile

C. sieberiana root powder (30 g) was soxhlet extracted with 300 ml. of petroleum ether (40-60°C) for 18 hours. The marc was air dried and extracted with a total of 600 ml of methanol. Each extract was concentrated to a syrupy mass separately using rotary evaporator at 50°C. under reduced pressure and dried in a desiccator. The yields were: petroleum ether, 0.40 g (1.3% w/w) and methanol; 6.31 g (21.03% w/w)

The petroleum ether and the methanol extracts, 100 mg each were dissolved in 6 ml of chloroform and 8 ml methanol respectively. These were spotted on a pre-coated silica gel plate, Merck, -055540001 using a capillary tube and developed in a solvent system, chloroform-ethyl acetate (3:1) and ethyl acetate: methanol: water: petroleum ether (8:2:0.3:0.3) respectively.

The plates were dried in an oven and sprayed separately with alcoholic potassium hydroxide and anisaldehyde (AS) to detect the presence of anthraquinone and other non-anthraquinone compounds.

RESULTS

Macromorphology of root

Cassia sieberiana root is highly branched. The outer and inner surfaces are brown and buff in colour respectively. The dried bark is curved with convex outer surface, figure 1.

Micromorphology

The transverse section (TS) of the root bark, figure 2 shows outermost layer of cork. There is a wide cortex made up of a ring of parenchyma cells, a continuous band of stone cells, stone cells in groups and fibres. The phloem shows large sieve tubes interspersed with phloem parenchyma and fibres.

The longitudinal section (LS) of the root bark, figure 3 shows the same tissue arrangement. The cell types and cell inclusions detected in the powdered root bark are given in figures 4, and 5.

Starch grains from *C. sieberiana* root powder are oval in shape with the length ranging from 60 to 180 μ (average 150 μ)and diameter ranging from 40 to120 μ (average 50 μ)

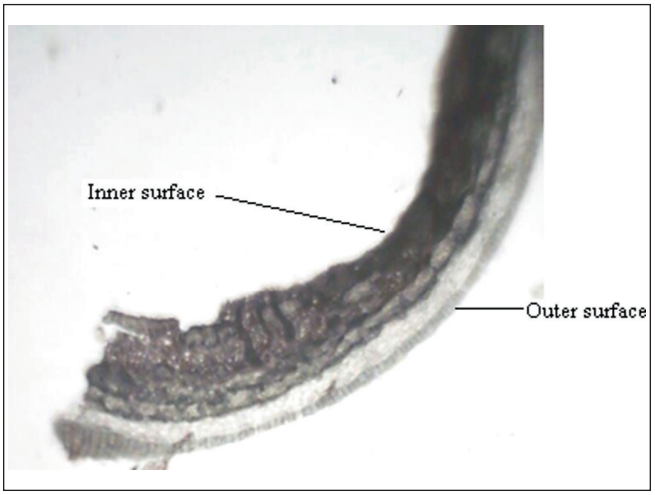


Figure 1: Curved appearance of dried *C. sieberiana* root bark

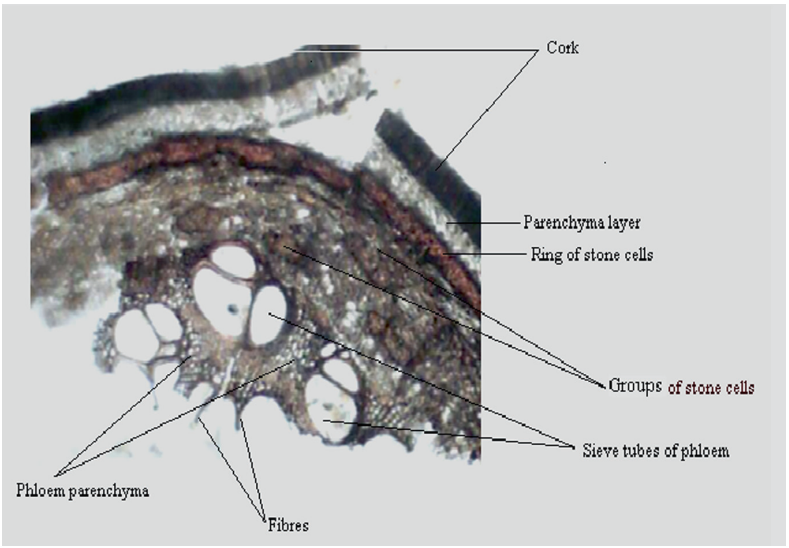


Figure 2: Tissues of *C. sieberiana* root bark

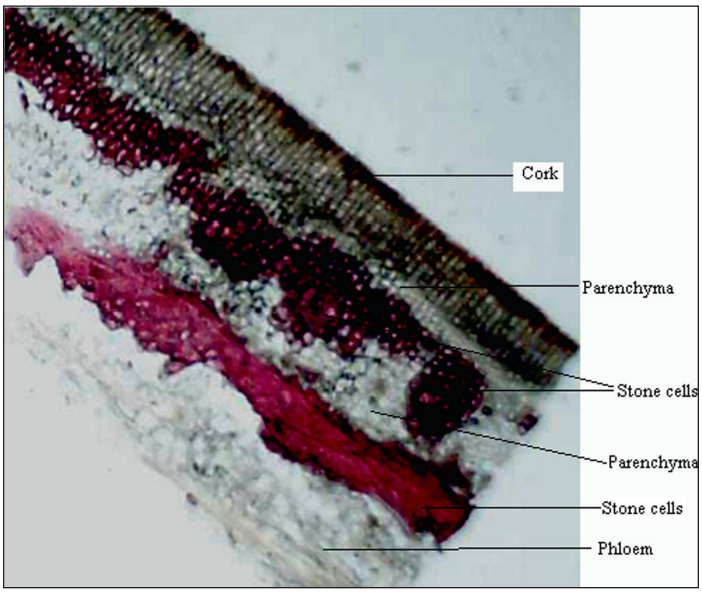


Figure 3: LS of *C. sieberiana* root bark

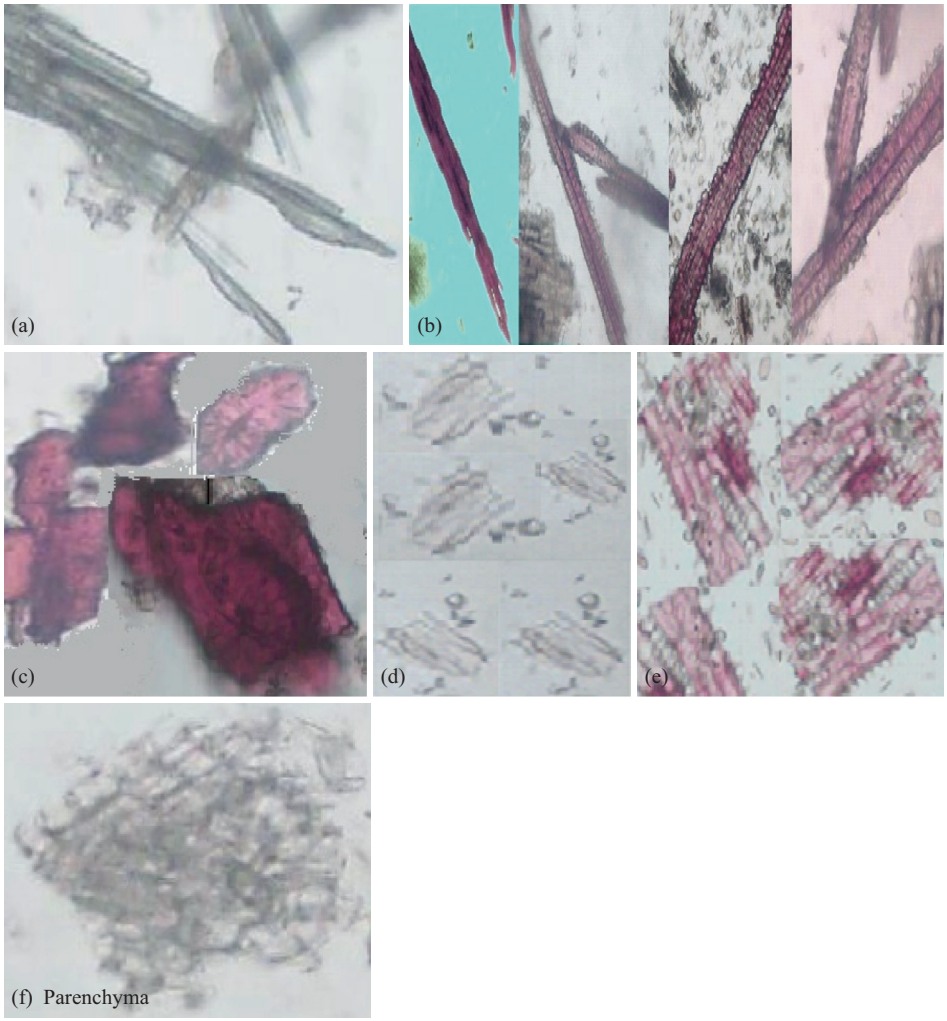


Figure 4: Some cell types from *C. sieberiana* root powder. a = unlignified fibres (×120), b = lignified fibres (×120), c = stone cells (×120), d = xylem vessels (×80), e = sieve tubes of phloem (×100), f= parenchyma (×100),
a, b, c and e were mounted in phloroglucinol in conc HCl, f was mounted in N/10 iodine

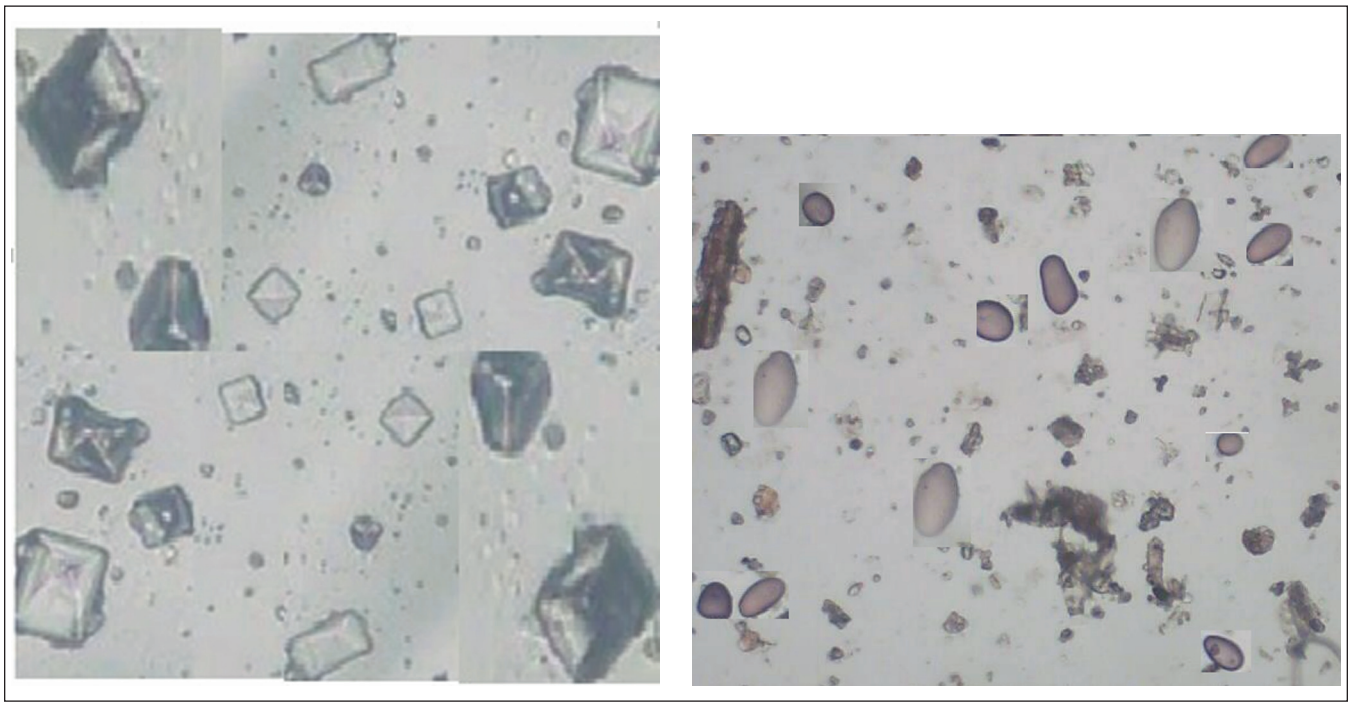


Figure 5: Cell inclusions from *C. sieberiana* root powder. a = calcium oxalate crystals (×100), b = starch grains (×50) mounted in N/10 iodine

Table 1: Results of physicochemical studies of *C. sieberiana* root

Total ash	7.43-8.37 (Average = 7.90% w/w)
Acid insoluble ash	4.80-5.50% w/w (Average = 5.15% w/w)
Ethanol (70%) extractive	14.28-14.30%w/w (Average = 14.38% w/w)
Chloroform-water extractive	15.99-19.98%w/w (Average = 17.99% w/w)
pH (1% w/v at 28°C)	4.88
Constituents detected in phytochemical screening	Anthraquinones, Saponin, Sterols, Steroidal glycosides, Tannins, Triterpenes

The calcium oxalate crystals are pyramid shaped with base diagonals measuring 40 to 160μ (average = 60 μ), figure 5

Physicochemical studies

Results of the physicochemical studies are presented in Table1.

Thin layer chromatography

The petroleum ether extract gave three anthraquinone compounds with R_f 0.69, 0.56 and 0.43 (Chloroform: Ethyl acetate (3:1), Temperature: 28-32°C.

DISCUSSION

Cassia sieberiana formulations are among the herbal preparations in popular traditional use as an analgesic due to its low or no observed side effects unlike that of allopathic pain killers: non-steroidal anti-inflammatory drugs (NSAIDs), that show a number of side effects including stomach ulcers, kidney failure and myocardial infarction especially in the elderly.[14][15] The plant has the potential for development into a standard analgesic medicine for medical use. Three anthraquinones compounds whose Rf values were fairly constant in the given solvent systems were detected and therefore can serve as tlc finger prints. Tambora *et al* also reported of the detection of anthraquinones in the plant.[7] Further work being undertaken to unravel the chemical nature of the compounds responsible for the observed analgesic property will further enrich the monograph on the plant and can also lead to possible synthesis of the active compounds

Conclusion The parameters established by this work, as summarised in the partial monograph could serve as the basis for *C. sieberiana* root standards.

ACKNOWLEDGEMENT

The authors would like to acknowledge the technical staff of Pharmacognosy and Pharmaceutical Chemistry Departments who at various stages got reagents and equipment ready for the work and also to Ms Ellen Adjubi who assisted in getting microscope slides ready for the work

REFERENCES

1. Evans WC. Trease and Evan's Pharmacognocy 13th Edition. ELBS/Baillère Tindall (1989).
2. Lawrence, D. R. and Bennett, P. N. Clinical Pharmacology ELBS with Churchill Livingston, Edinburgh (1995).
3. Carr I. The far beginnings, a brief history of medicine. Health Sciences Library, University of Manitoba [serial on the Internet] 1997 August [cited 2009 Aug30]. Available from: [http://umanitoba.ca/faculties/medicine/history/salerno/index.htm]
4. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanahoun E, Ahyi M RA, Ekpere JA *et al*,. Floristic Studies in Ghana. Scientific, Technical and Research Commission, OAU/STRC (2000).
5. Sy GY, Fall AD, Diatta W, Gueye M, Khady B, Bassène E. *et al* Analgesic and anti-inflammatory activity of aqueous root extract of *Cassia sieberiana* D. C. (Caesalpiniaceae) African Journal of Pharmacy and Pharmacology 2009; Dec 3(12):651-653.
6. Gabriëlla HS and Ameenah GF Plant Resources of Tropical Africa Medicinal plants, [serial on the Internet] 2010 Nov [cited 2010 Nov 10]. Available from [http://www.amazon.com/sieberiana]
7. Tamboura HH, Bayala B, Lompo M, Guissou IP and Sawadogo L. Ecological distribution, morphological characteristics and acute toxicity of aqueous extracts of *Holarrhena floribunda* (G. DON) DURAND & SCHINZ, *Leptadenia hastata* (PERS.) DECNE and Cassia sieberiana (D C) used by veterinary healers in Burkina Faso. African J. Of Traditional, Complementary and Alternative Medicines [serial on the Internet].2005 Aug [cited 2010 Jun 15] 2(1) Available from*:* [http://www.bioline.org.br/request?tc05004]
8. Watson L, and Dallwitz M J The Families of Flowering Plants [serial on the Internet] 2000 August [cited 2008 August18]. Available from: [http://biodiversity.uno.edu/delta2000]
9. Duwiejua M, Panyin A B, Weremfo A, Woode E and Ansah C. Actinociceptive activity of the ethanol extract of the root of *Cassia sieberiana. J. of Pharmacy and Bioresources* [serial on the Internet]. 2007 Jun [cited 2008 Aug 12] 4(2):49-58.
10. Sam GH, Mensah M L K,Sarpong K and Duwiejua M. Antispasmodic activity of methanolic extract of *Cassia sieberiana* root D.C. [Caesalpiniaceae]. Proceedings of the 3rd Conference of West African Network of Natural Products Research Scientists, 2008 August. 6-9 Ouagadougou, Burkina Faso.
11. Bulner SH. Some Arguments against the Standardization of Herbalists [serial on the Internet] 2002 August [cited 2008 August18] Available from [http://www. gaianstudies.org/articles.htm]
12. Council of the Pharmacopoeial Soc. Of Gt Britain. British Pharmacopoeia Biddles Ltd 1990 Gt Britain 1990.
13. British Herbal Medicine Association; British Herbal Pharmacoepoeia 1990 Guildford and King's Lynn, GT 1990.
14. Katzung B G. Basic and Clinical Pharmacology. 5th Ed Lange Med. Pbs; Calif; 1982.
15. American College of Gastroenterology; The dangers of Aspirin and NSAIDs 2010 Bathesda 2011.

Pharmacognostic evaluation of the rhizomes of *Curcuma zedoaria* Rosc.

Sharad Srivastava, Shanta Mehrotra and AKS Rawat*

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, (CSIR), Lucknow-226001, India

ABSTRACT

Curcuma zedoaria Rosc. (Family-Zingeberaceae), commonly known as '*yellow zedoary*' is used in India system of medicine since time immemorial. The plant is found well in eastern Himalayas, Chittangang, Bengal, Kerala, Konkan and often cultivated throughout India. It is aromatic, pungent, bitter and useful in flatulent colic and debility of the digestive organs and also used as an ingredient in bitter tincture of zedoary and anti-periodic pills. A paste of rhizome is useful externally for cuts, wounds, itching and in sprains. A detailed phytochemical evaluation of its rhizome showed moisture content 83.22%, total ash 6.64%, acid insoluble ash 0.64%, alcohol soluble extractives 15.53%, water soluble extractives 18.96%, sugar 12.51% and starch 15.70%. A study of its volatile content also has been done indicating 2.8% of total volatile oil. These findings will be very useful for the identification of the species which may be useful to pharmaceutical industries for the quality control of the commercial samples.

Key words: *Curcuma zedoaria*, HPTLC, Pharmacognosy, Standardization.

INTRODUCTION

Curcuma zedoaria Rosc. (Zingeberaceae), commonly known as '*yellow zedoary*' is aromatic, pungent, bitter and useful in flatulent colic and debility of the digestive organs and also used as an ingredient in bitter tincture of zedoary and anti-periodic pills, a paste of rhizome is useful externally for cuts, wounds, itching and in sprains.[1-4]

Rhizomes are employed in Asian and many other countries, including Brazil, for the treatment of several ailments, such as cervical cancer,[5,6] hepatitis, inflammations[7] and dolorous processes.[8] Several studies have confirmed and also extended most of the mentioned popular uses of this plant. In this context, several workers were demonstrated its antifungal,[9] antiulcer,[10] antimutagenic,[11] hepatoprotective[12] and cytotoxic[6] properties. It is well-documented that its main active principles are terpenoids, especially sesquiterpenoids[5,6,13,14] which also are produced by cultured cells.[15] *C. zedoaria* is also commonly used in medicine but with high starch content.[16] Phytochemical analysis and analgesic properties of *Curcuma zedoaria* grown in Brazil has also been done.[17]

Address for correspondence:
Phone: +91 522 2297816; Fax: +91 522 2207219;
E-mail: pharmacognosy1@rediffmail.com

DOI: 10.5530/pj.2011.21.3

Traditionally, the dried rhizome of *C. zedoaria* is selected to make drinks or to be extracted as medicine. It has been reported that the boiling water extracts of *C. zedoaria* had a moderate antimutagenic activity against benzo[α]pyrene.[11] Some hepatoprotective sesquiterpenes have been isolated from the aqueous acetone extracts of *C. zedoaria* rhizome. The major sesquiterpene compounds, including furanodiene, germacrone, curdione, neocurdione, curcumenol, isocurcumenol, aerugidiol, zedoarondiol, curcumenone and curcumin, were found to show potent protective effect on d-galactosamine/lipopolysaccharide-induced liver injury in mice.[18]

Although the drug is fairly important and has good economics but no pharmacognostical work have been done in details. Therefore the present study had been done to document its detailed pharmacognostical information which will be utilized by the industries for the authentication and quality control of this drug.

EXPERIMENTAL

Plant material

The plant material was collected from field near Palghat area of Kerala, India, authenticated and lodged in Institute's Herbarium [LWG 221248, 1999] and the rhizomes were preserved in 70% ethyl alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera.[19]

Physico-chemical and phytochemical assays

Physico-chemical and phytochemical studies has been done from the shade dried powdered material according to the recommended procedures.[20-22] The behavior of the powdered drug with different chemical reagents was also studied as per methods described.[23,24]

HPTLC studies

The HPTLC analysis was carried out on precoated silica gel 60 F_{254} Merk glass plates of 20 × 10 cm with the help of Camag Linomat- IV applicator and eluted the plate at room temperature in solvent system Chloroform:Ethanol:Acetic acid (95:5:0.1).

RESULTS AND DISCUSSION

Macroscopic characters of the rhizome

The primary rhizome or rootstock is almost conical or top shaped. Attached to the primary rhizome are several sessile finger shaped lateral branches. The rhizome as well as its branches has an acrid or pungent taste and a distinct aromatic, camphoraceous smell. (Plate-1)

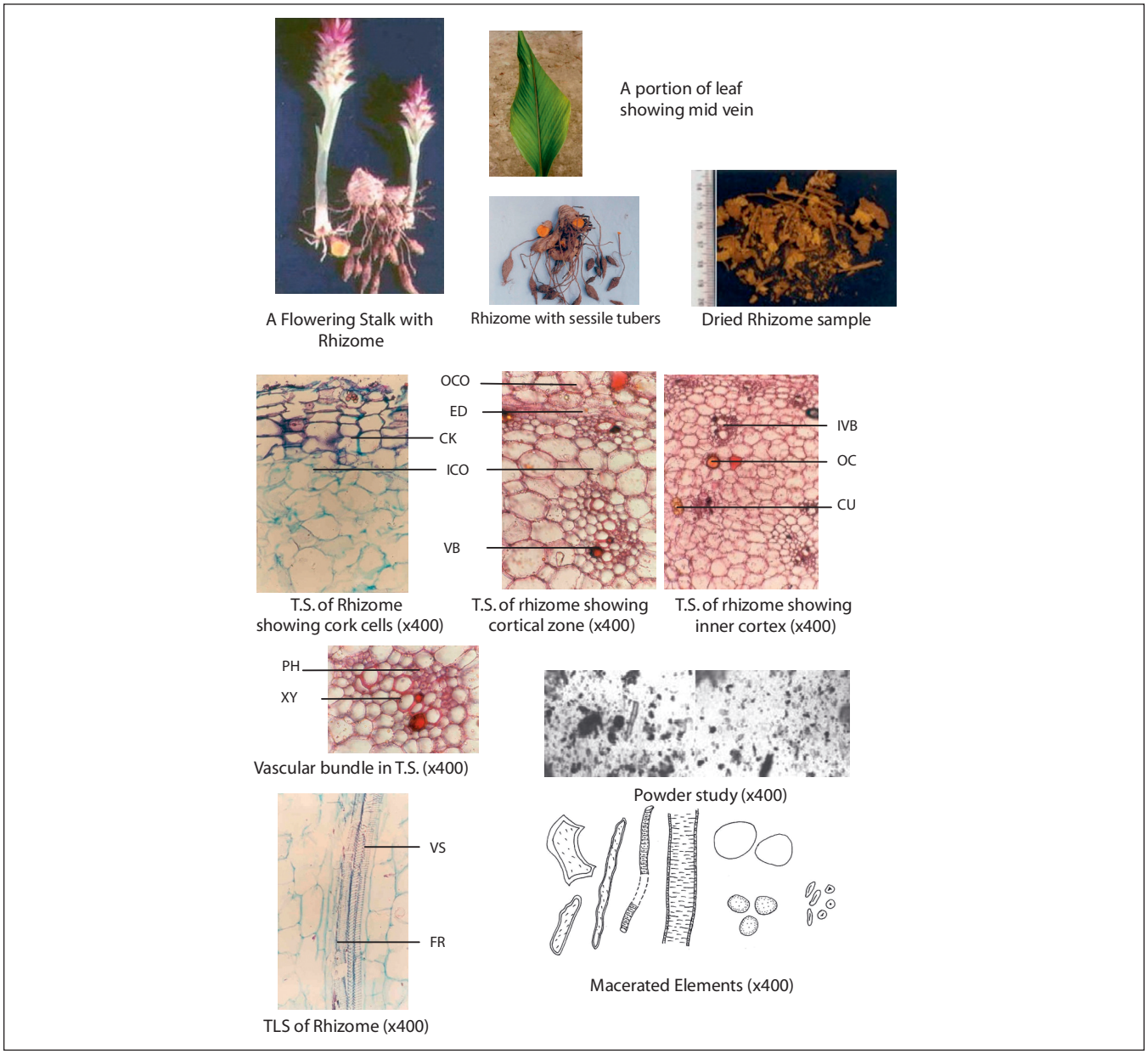


Plate-1: Macro and Microscopic characters of the rhizome of *Curcuma zedoaria* Rosc.

Abbreviations

ICO, Inner cortex; CK, Cork cells; IVB, Inner vascular bundle; OVB, Outer vascular bundle; CO, Cortex; FR, Fibre; ST, Starch; VS, Vessels; XY, Xylem; ED, Endodermis; PR, Pericycle.

Microscopic characters of the rhizome

Epidermis, the outermost layer composed of rectangular tangentially elongated cells. In older rhizomes the epidermis is replaced by cork composed of 7 to 10 rows of typical rectangular to tangentially elongate thin walled cells. The ground tissue is differentiated into two regions, the outer cortex and the inner cortex by a distinct endodermis. The cortical ground tissue just beneath the cork contain yellowish contents i.e. curcumin. Almost all the parenchymatous cells forming the ground tissue are densely packed with starch grains. The starch grains are simple, comparatively big, flattened, rectangular or ovoid and possess a slight projection at one end. The striations on the grains are numerous. The endodermis is composed of a row of thin walled elongated cells with their radial walls slightly thickened. The cell layer within the endodermis also has tangentially elongated cells but narrower and some of them contain very small oblong starch grains. The cortical bundles as well as those within the stele are similar in structure. Bundles with a single vessel are very rare. Most of the bundles just within the endodermis are small. These contain only 2 to 5 xylem vessels. Each vascular bundle has got a sheath of small sized parenchyma cells, which completely encircle it. The cells forming the sheath, as is the case in the endodermal cells do not contain any starch (Plate-1).

Quantitative microscopy

On maceration, the vessels (744.939 × 15.829 µm) with annular and spiral thickenings are observed. Tracheids with bordered pits measuring 643.453 × 15.123 µm are also clearly discernable (Plate-1).

Study powdered rhizome

Powder light yellow, sweet, strong pungent, aromatic odour; shows fragments of storied cork, xylem vessels with reticulate thickenings, lignified xylem fibres, oil cells, patches of parenchymatous cells filled with starch grains which are oval-ellipsoidal, sometimes polygonal in shape, 10 to 60 µm, simple, hilum circular or a 2 to 5 rayed cleft, lamellae distinct and concentric (Plate-1).

The behavior of the powdered drug with different chemical reagents has been shown in the Table 1. From the above studies rhizome can easily be differentiated on the basis of organoleptic characters for example the odour and taste of rhizome is quite characteristic and is aromatic with pungent taste. On microscopical examination rod shaped starch grains and fibres are observed in the rhizome. Similarly numbers of curcumin containing cells which are yellow in colour are also high in the rhizome.

Physico-chemical studies

Physicochemical values *viz*. percentage of moisture, total ash, acid insoluble ash, alcohol and water-soluble extractives are observed. The total ash and acid insoluble ash, which are considered to be an important and useful parameter for detecting the presence of inorganic substances like silicate ion, it was found 6.64% and 0.64% respectively. Similarly the alcohol and water-soluble extractives, which are indicators of the total solvent soluble components, are 15.53% and 18.96% respectively. Likewise the essential oil, which is an important parameter for identification and authentication it was found to be 2.8% (Figure 1).

Successive Soxhlet extraction from non-polar to polar solvents *viz*. hexane, chloroform, acetone, alcohol and water were also carried out. It is interesting to note that *C. zedoaria* rhizome possessed an exceptionally high amount of acetone extractives i.e. 38.467%, which may be due to the higher percentage of curcumin which is purely soluble in acetone this is also comparable to amount of curcumin in *C. longa* (Figure 2). The preliminary phytochemical screening of different successive extractives is recorded in Table 2.

Table 1: Fluorescence powder study of *C. zedoaria* rhizome

S. No.	Treatment	Day light	UV-254 nm	UV-366 nm
1.	Powder (P) as such	Yellowish Brown	Brown	Brown
2.	P. + Nitro-cellulose in amyl acetate	Florescent Yellow	Florescent Yellow	Yellow
3.	P. + N. NaOH in water	Brick Red	Dark Brown	Brown
4.	P. + 1N NaOH + Nitro-cellulose in acetate	Brick Red	Brown with Yellowish Tinge	Black
5.	P. + 1N HCl + Nitro-cellulose in amyl acetate	Florescent Yellow	Florescent Yellow	Yellow
6.	P. + 1N NaOH in Methanol	Brick Red	Brown with Yellowish Tinge	Black
7.	P. + 50% KOH	Brick Red	Brown with Yellowish Tinge	Black
8.	P. 1N HCl	Brown	Light Brown	Black
9.	P. + 50% H_2SO_4	Black	Dark Brown	Black
10.	P. + 50% HNO_3	Brown with Yellowish Tinge	Brown with Greenish Tinge	Brown with Violet Tinge
11.	P. + Conc. HNO_3	Muddy Yellow	Brown with Greenish Tinge	Black
12.	P. + Acetic acid	Light Brown	Light Brown	Black
13.	P. + Conc. H_2SO_4	Black	Black	Black
14.	P. + Iodine water	Black with Greenish Tinge	Black with Greenish Tinge	Black

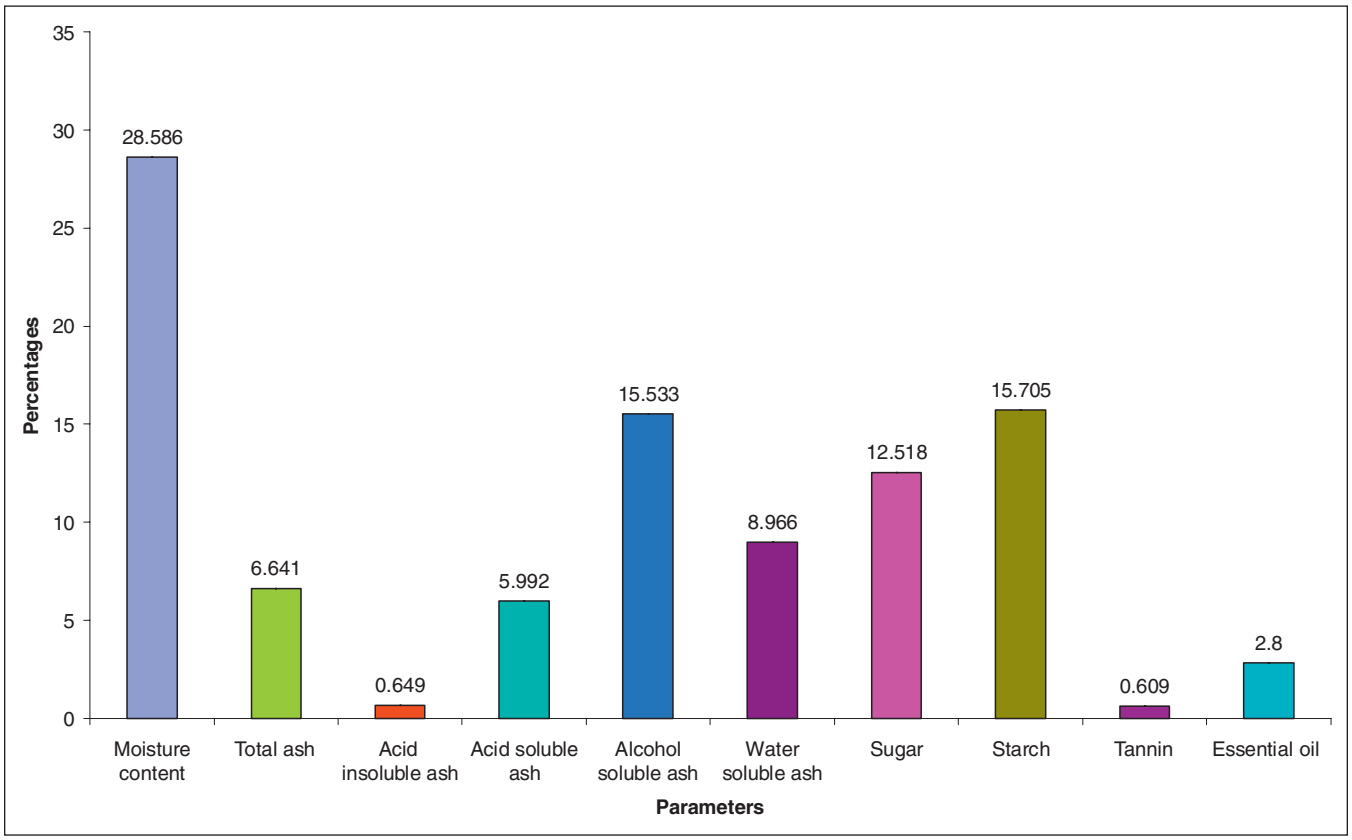


Figure 1: Physicochemical values of *Curcuma zedoaria*

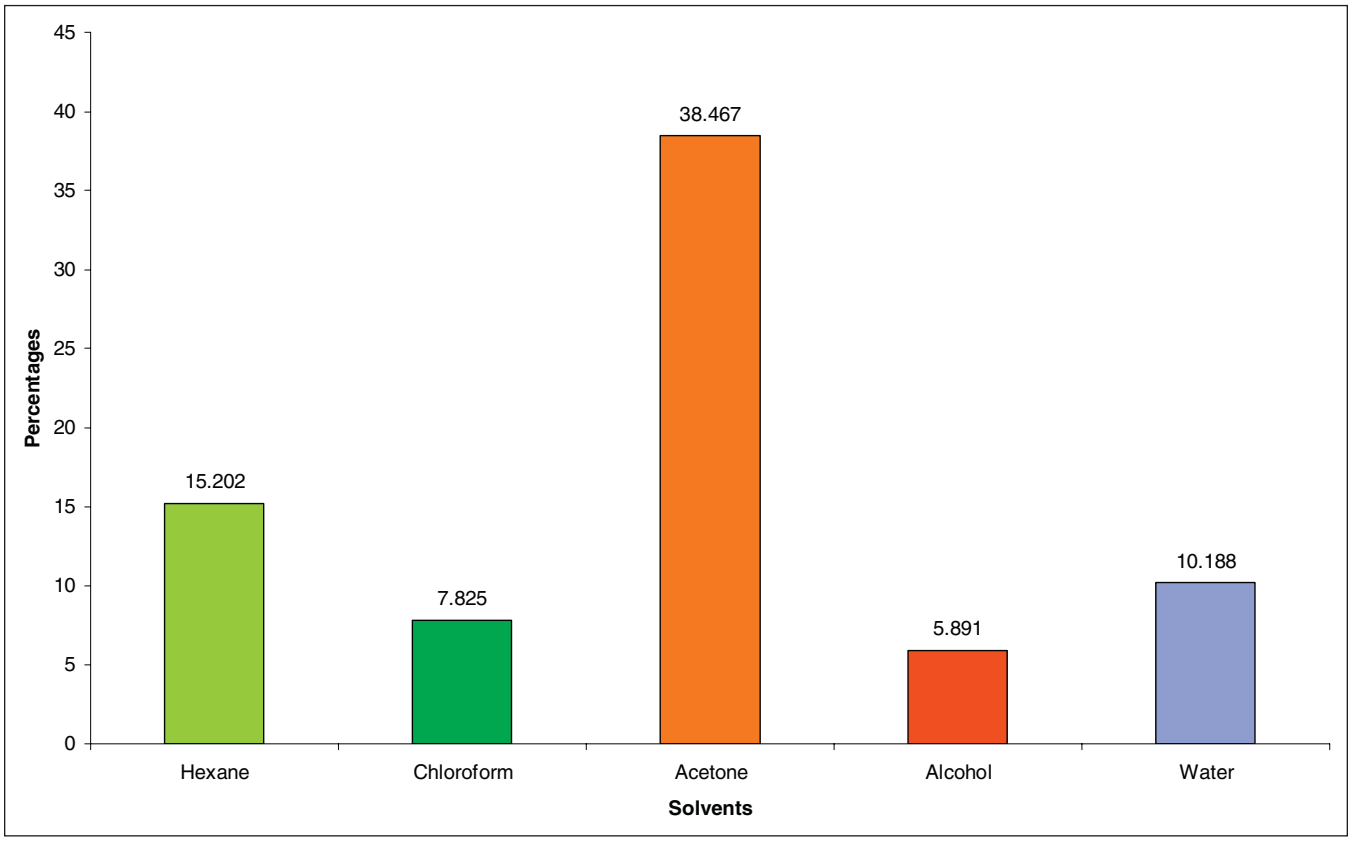


Figure 2: Successive soxhlet value of *Curcuma zedoaria*

HPTLC studies

A densitometric HPTLC analysis was also performed for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. The HPTLC analysis was carried out on precoated silica gel 60 F_{254} Merk glass plates of 20 × 10 cm with the help of Camag Linomat-IV applicator and eluted the plate at room temperature in solvent system Chloroform:Ethanol:Acetic acid (95:5:0.1). The bands in the sample are obtained at R_fs 0.17, 0.43, 0.66, and 0.84, which can be used as identifying markers.

The Curcumin was identified at R_f 0.84. (Plate-2)

Table 2: Phytochemical screening of different extracts of *C. zedoaria* rhizome

Extractive	Triterpe-noids & steroids	Saponins	Flavanoids	Tannins	Reducing sugars	Resins	Glycosides	Alkaloids
Hexane	+	–	–	–	–	–	–	–
Chloroform	–	–	–	–	–	–	–	–
Acetone	+	–	–	–	–	–	–	–
Alcohol	–	–	–	–	–	+	–	+
Water	–	+	–	–	–	–	–	–

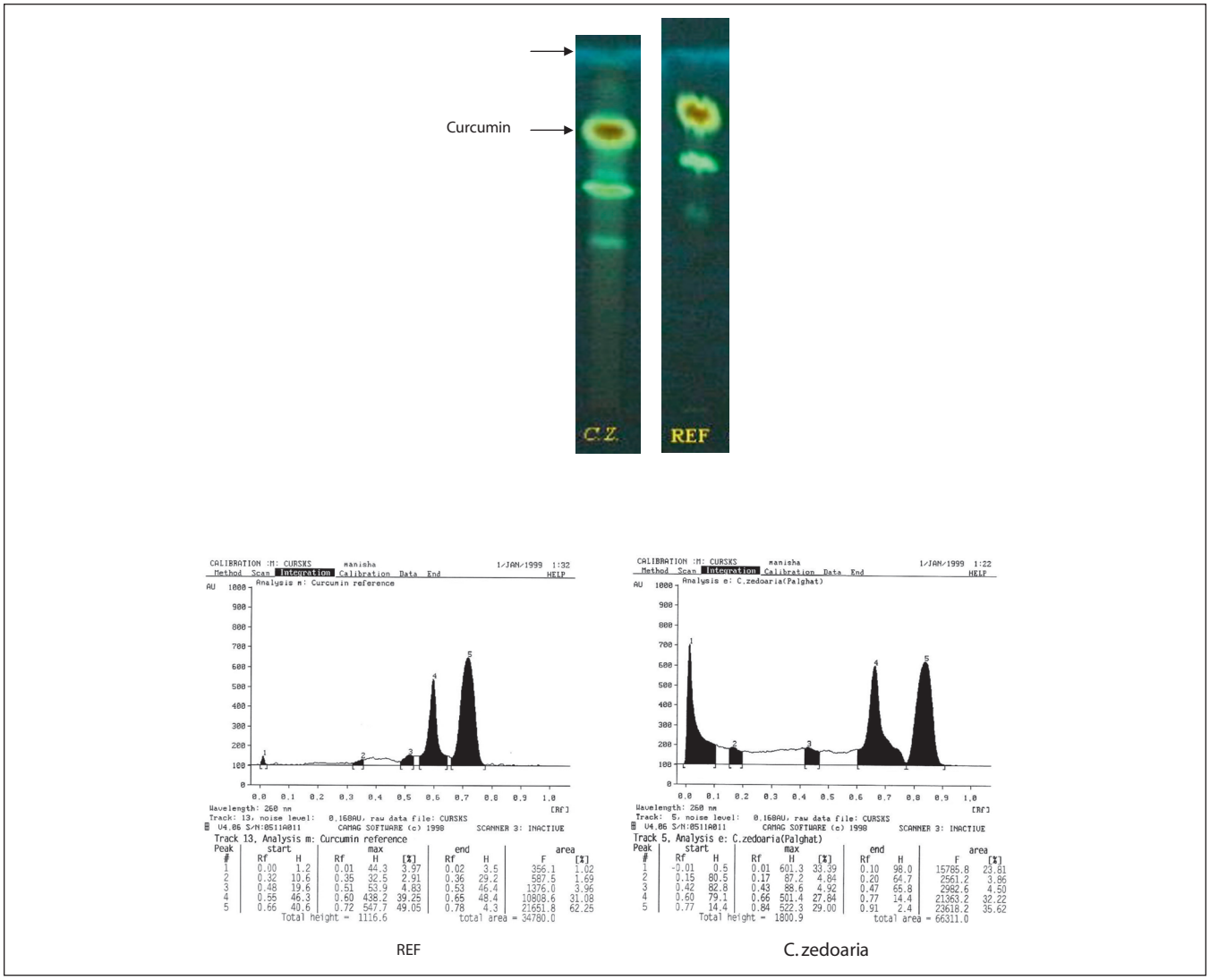


Plate-2: HPTLC Profile of *Curcuma zedoaria* Rosc. and reference sample (Under UV- 366)
1. Reference sample of Curcumin
2. HPTLC profile of Curcuma zedoaria *Rosc.Rhizome*

Abbreviations
REF, curcumin. reference; CA, *Curcuma zedoaria* Rosc.

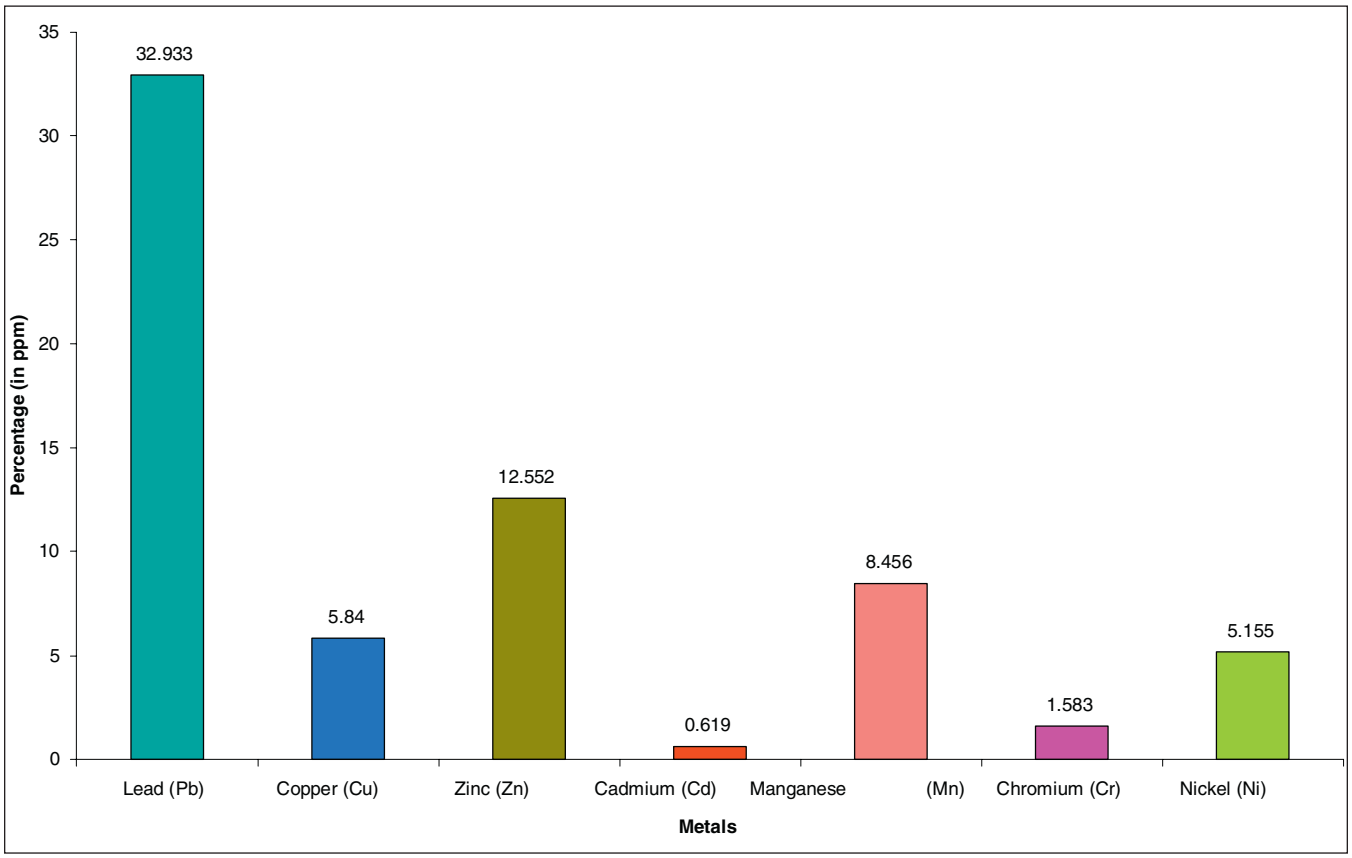


Figure 3: Heavy Metal studies in *Curcuma zedoaria* rhizome

Heavy metal studies

The various heavy metals viz. Pb, Cd, Co, Mn, Cu, Zn and Hg concentrations was also estimated in the samples and all the metals are found within the permissible limits as prescribed by the WHO. (Figure 3)

CONCLUSION

Thus on the basis of aforesaid studies, it can be concluded that the above parameters are very useful for the identification of the species which may be useful to pharmaceutical industries for the quality control of the commercial samples.

ACKNOWLEDGEMENTS

The authors are thankful to Director, NBRI for providing all the facilities to conduct this research work.

REFERENCES

1. Watt, G. A Dictionary of the Economic products of India, vol. II, Kolkatta, 1889, pp. 652.
2. Kirtikar KR, Basu BD. Indian Medicinal Plants, vol. IV, L. M. Basu Pub. Allahabad, 1933, pp 2422.
3. Nadkarni, A.K. K M Nadkarni's Materia Medica, Dhootpapeshwar Prakasan Ltd., Mumbai, 1954, 412-413.
4. Chopra RN, Chopra IC, Handa KL, Kapoor LD. Indigenous drugs of India, U N Dhur and Sons, Kolkata, 1958, pp 503.
5. Ma X, Yu X, Han J. Application of off-line supercritical fluid extraction-gas chromatography for the investigation of chemical constituents in Curcuma zedoaria. *Phytochem Anal,* 1995;**6:**292-96.
6. Syu WJ, Shen CC, Don MJ, Ou JC, Lee GH, Sun CM. Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zedoaria. J Nat Prod,* 1998; **61:**1531-34.
7. Tang, W. and Eisenbrand, G. Chinese drugs of plant origin: chemistry, pharmacology and use traditional and medicine. 1992. Springer Verlag, Berlin.
8. Teske, M. and Trentini, A.M.M. Compêndio de Fitoterapia, Ed. Herbarium, Colombo-Paraná, 1995. Brazil.
9. Gupta SK, Banerjee AB, Achari B. Isolation of ethyl methoxy cinnamate, the major antifungal principle of *Curcuma zedoaria*. *Lloydia,* 1976; **39:**218-222.
10. Sakai K, Miyazaki Y, Yamane T, Saitoh Y, Ikawa C, Nishihata T. Effect of extracts Zingiberaceae herbs on gastric secrection in rabbits. *Chem Pharm Bull,* 1989; **31:**215-17.
11. Lee H, Lin JY. Antimutagenic activity of etracts from anticancer drugs in Chinese medicine. *Mut Res,* **1988; 204:**229-34.
12. Rana AC, Avadhoot Y. Experimental evaluation of hepatoprotective activity of *Gymnema sylvestre* and *Curcuma zedoaria*. *Fitorerapia,* 1992; **63:**60-63.
13. Hikino H, Agatsuma K, Takemoto T. Sesquiterpenoids- structure of isocurcumenol. *Chem Pharm Bull,* 1968; **17**:959-60.
14. Shiobara, Y., Asakawa, Y., Kodama, M., Yaduda, K. and Takemoto, T. (1985). Curcumenone, curcumanolide A and curcumanolide B, three sesquiterpenoids from *Curcuma zedoaria*. *Phytochemistry,* 1985; **11:**2629-33.

15. Sakui N, Kuroyanagi M, Ishitobi Y, Sato M, Ueno A. Biotransformation of sesquiterpenes by cultured cells of Curcuma zedoaria. *Phytochemistry,* 1992; **31:**143-147.
16. Matsuda H, Morikawa T, Ninomiya K, Yoshikawa M. Hepatoprotective constituintes from zedoariae rizoma: absolute stereostructures of three new carabrane-type sesquiterpenes, curcumenolactones A, B, and C. *Biorganic and Medicinal Chemistry* 2001; **9:**909-916.
17. Navarro D, de Fátima de Souza MM, Neto RA, Golin V, Niero R, Yunes RA, Delle Monache F., Cechinel Filho V. Phytochemical analysis and analgesic properties of *Curcuma zedoaria* grown in Brazil. *Phytomedicine,* 2002**; 9:**427-32.
18. Matsuda H, Ninomya K, Morikawa T. Inhibitory effect and action mechanism of sesquiterpenes from Zedoariae rhizome on D-galactosamine lipolysaccharide-induced liver injury. *Bioorg Med Chem Lett.* 1988; **8:**4339-44.
19. Josansen, D.A. Plant Microtechnique, **1st** Edn., 182 MC Graw Hill Book Co. Inc., 1940, New York and London.
20. Peach K. and Tracy MV. Modern Methods of Plant Analysis (Heidelberg Springer) 3rd and 4th vol., 1955.
21. Anonymous. Indian Pharmacopoeia, 1965, 2nd Edn. Govt. of India; New Delhi.
22. Anonymous. Official methods of Analysis (AOAC), 1984, 4th Edn. Association of Official Chemists, Inc. USA.
23. Chase CR, Pratt RJ. Fluorescence of Powdered vegetable drugs with particular reference to development of a system of identification. *J Amer Pharmacol Assoc. 1949;* **38:**324-31.
24. Kokoski J, Kokoski R, Slama FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J Amer Pharmacol Assoc. 1958;* 47:75.

Pharmacognostical and Phytochemical Evaluation of Leaves of *Cissampelos pareira*

Arti Gupta*[1], Sonia Pandey[2], D.R Shah[3], N.R. Seth[4], J.S. Yadav[5]

[1]Maliba Pharmacy College, Gopal Vidhyanagar,Tarsadi, Bardoli,South Gujarat, India. [2]Department of Pharmacognosy, Saurashtra University, Rajkot, North Gujarat, India. [3]Department of Pharmaceutics, Vidyabharati trust College of Pharmacy Umrakh, Bardoli, South Gujarat, India

ABSTRACT

In ethanomedicinal practices the the tradicinal healers use the roots of *Cissampelos pareira* in the treatment of various ailments related to urinary problems, skin infections,in tumor inhibitor activity,antibacterial, antimalarial, diuretic activity and anticonvulsant activity etc. Macro- and microscopical characters, behaviour of drug on treatment with different chemical reagents, fluorescence analysis, extractive values, ash values and preliminary phytochemical tests were carried out to study the distinctive features of the drugs. Such parameters provide basis for standardisation/characterisation of genuine drug. A serious limitation encountered in the use and research of traditional medicine is the lack of standardisation and quality control of raw material forming the drug. The ultimate objective of the pharmcognostic investigation is identification of the genuine crude drug and determining the extent of adulteration/substitution, if any. Advancement in recent years in pharmacognosy, phytochemistry and physicochemical techniques can be of immense value in removing this major shortcoming of traditional medicine. These techniques can be utilised for correct botanical identification of plants. The details of organoleptic, macro- and microscopic characters, various evaluative parameters, fluorescence analysis, results of preliminary phytochemical analysis established in the present study will facilitate in identifying the g drug from any substitute or spurious samples and will also be useful in preparation of monographs on these plants.

Key words: *Cissampelos pareira* root, Standardization, Pharmacognosy, Physico-Chemical

INTRODUCTION

C. pareira of the family Menispermaceae are commonly known as Patha in Ayurveda and have been used for the treatment of fever, urinary problems and skin infections.[1] *C. pareira* is found very common in semi dry forests of tropics.[2] Various alkaloids and different pharmacological activities of these plants have been reported. Hydrocolloids,[3] Cissampeloflavone,[4] A tropone-isoquinoline alkaloid, pareitropone,[5] Bisbenzylisoquinoline alkaloids, cissamperine with tumor inhibitor activity,[6] trandrine,[7] tropoloisoquinoline alkaloids such as pareirubrine A and B with antileukemic activity[8] have been isolated from *C. pareira Var. hirsuta*. Plant extracts were tested for Antipyretic activity,[9] Chemomodulatory influence of Hirsuta on Gastric cancer and antioxidant system in experimental animal,[10] anti-inflammatory activity,[9] Immunomodulatory activity,[10] antifertility activity, antinociceptive, antiarthritic activity, antibacterial activity,[11] antimalarial activity,[12] diuretic activity,[13] hypoglycemic activity[14] and anticonvulsant activity.[15] A few ethnobotanical reports on treatment of fever,[16] gastrointestinal tract disorders[17] were also investigated.

Address for correspondence:
E-mail: aarticognosy@yahoo.co.in; aarti137@rediffmail.com

DOI: 10.5530/pj.2011.21.4

MATERIALS AND METHODS

Plant material

The fresh leaves of *C. pareira* were collected in the month of April, 2008 from balsar, Dist. Balsar , India and was authenticated by Minoo H. **Parabia**. Professor, Department of Bioscience. **Veer Narmad South Gujarat University**, Surat. Voucher specimen (No: MPC/13032010/02) has been deposited in the Department of Bioscience. **Veer Narmad South Gujarat University**, Surat India. Collected fresh leaves were washed, shade dried and was pulverized with a mechanical pulverized for size reduction. It was then passed through # 60 and the fine power was collected and was used for the experiment for powder microscopy and preparation of extract. The fresh leave sample was used for microscopy identification. The powder of dried leaves was used for the determination ash values, extractive values and phytochemical investigations. All chemicals and reagents used for testing were analytical grade obtained from Atul Chemicals Baroda (India).

Pharmacognostical studies

Morphological studies were done by using simple microscope to determine the shape, apex, base, margins taste and odour of leaves. microscopic studies were done by preparing a thin hand section of mid rib and the lamina region of *C. pareira*. The section was cleared with chloral hydrate solution and was stained as per protocol. Histochemical reactions were applied with concentrated hydrochloric acid and phloroglucinol and were mounted in glycerin for the identification of lignified elements, iodine solution for identification of starch grains, ruthenium red for mucilage, 60% sulfuric acid for calcium oxalate crystals and ferric chloride for the phenolic compounds in the powdered bark by the reported method. A part of quantitative microscopy, stomatal number, stomatal index, was determined by using fresh leaves of plant.

Microscopy

Plant material were preserved in a mixer of solvent containing formalin, acetic acid and alcohol 70% (v/v) for histological studies, transfer section of the leave was taken using the microtome and stain with different stains.[18] Microphotograph of the section were made using olympus BX 40 microscope attached with Olympus DP 12 digital camera.

Physicochemical Constants

Physico-chemical constants such as percentage of total ash, water soluble ash, water and alcohol soluble extractives and los on drying (LOD) were calculated as per the Indian Pharmacopoeia.[19] Total phenolic content in a crude drug was estimated according to the method given by singleton and rosy.[20]

Phytochemical Screening

For preliminary phytochemical studies, 65 gm of powdered material extracted in soxhelet apparatus with alcohol, obtained extract were dried and weighed, the presence of various phytoconstituents steroids and triterpenoids (liberman and burchard test, alcohol (dragandroff's test) tannin and ferric chloride test) flavonoid (shinoda test), Sugar (fehling solution tesst), aminoacid (ninhydrin test) were detected by usual method prescribed in standard text.[21,22]

RESULTS

Macroscopic characters of leaves

Organoleptic characters

Colour of leaves are greenish on outer side and grayish underneath.Size and shape: 3-9 × 5-7cm, Cordate. Apex of the leaf is variable, normally it is obtuse or Emarginate. Taste is bitter. Odour is Slightly aromatic .The leaf shows entire margin,unequal bases, finely palmate venation and peteolated. (Figure 1)

Microscopy

a) Transverse section of leaf (Figure 2)

It is a dorsiventral leaf. Following tissues are present in midrib and lamina: **Midrib** (Figure 2a): Section passing

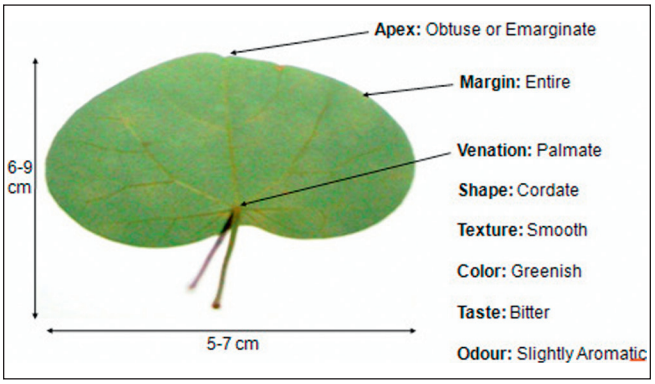


Figure 1: Leaves of *Cissampelos pareira Var. hirsuta*

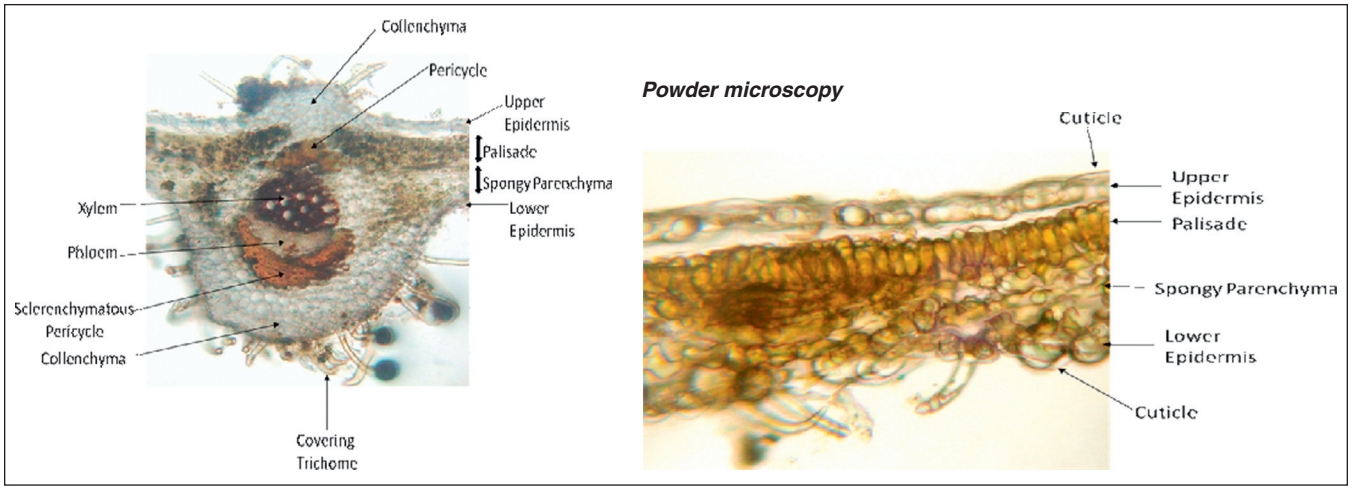


Figure 2: Leaf structure of *Cissampelos pareira Var. hirsuta*. (a) transverse section through midrib; (b) Transverse section through lamina

through midrib represents convex shape. Midrib shows 5-6 layers of collenchyma below the upper epidermis. It shows collateral type of vascular bundles. Distinct phloem tissue can be seen on the ventral surface and well developed xylem tissue towards the dorsal surface of the midrib. Xylem shows presence of tracheids, xylem parenchyma, protoxylem, and metaxylem towards lower periphery. Tracheids are tubular and elongated, while xylem vessels are reticulate. Thick walled non-lignified phloem follows the xylem. Phloem parenchyma is present in the form of a broad patch with scattered phloem fibres. The vascular bundle is encircled with pericyclic layer. The pericyclic layer is composed of 4-6 layers of lignified, thick walled cells. Pericycle is covered with parenchymatous cells followed by presence of 2-3 layered collenchyma above lower epidermis. Leaf shows presence of cluster of calcium oxalate crystals and starch grains. It also exhibits presence of secretory cavities. **Lamina** (Figure 2b) The lamina of the leaf shows upper epidermis, mesophyll and lower epidermis. Upper epidermis is composed of flat single layer of rectangular cells. Mesophyll is differentiated into palisade tissue and spongy parenchyma. Palisade cells are single layered, elongated and compactly arranged while spongy parenchyma which is composed of polygonal cells irregularly arranged and fill the entire space of lamina. Lower epidermis consists of single layer of rectangular cells, identical to upper epidermis. Both layers of epidermis are covered with a thick cuticle. Results of various histochemical reactions are given in Table 4. Different leaf constants are tabulated in Table 3.

Table 1: Fluorescence analysis of powdered leaf of *Cissampelos pareira*

Sr. No.	Samples	Color in	
		Day light	Uv light
1.	Powder	Green	Green
2.	Powder + NaOH in Methanol	Greenish brown	Dark green
3.	Powder + NaOH in water	Greenish brown	Dark green
4.	Powder + 1 N HCl	Brown	Dark green
5.	Powder + 50% HNO_2	Brown	Dark green
6.	Powder + 50% H_2SO_4	Brown	Dark green

Table 2: Physicochemical evaluation of the crude drugs of *Cissampelos pareira*

Sr. No.	Standardization parameters	% W/W
1.	Total ash	15.00
2.	Acid insoluble ash	1.76
3.	Water soluble ash	8.89
4.	Alcohol soluble extractive values	2.35
5.	Water soluble extractive value	4.79
6.	Loss on drying	13.33

Table: 3 Quantitative analysis of leaf constants of *Cissampelos pareira*

Leaf constants	Values	
	Upper surface	Lower surface
Stomatal number	10 per sq mm	13 per sq mm
Stomatal index	18.51	25.00

b) Powder characteristics (Figure 3)

Fibers are few and lignified. Numerous anisocytic or cruciferous stomata meaning thereby that the cells surrounding the stomatal pores are unequally arranged and cannot be differentiated from other epidermal cells.

Physico-chemical parameters

The percentage of total ash, acid-insoluble ash, water soluble Ash and different extractives are tabulated in Table 1.

Table 4: Histochemical color reactions of *Cissampelos pareira*

Reagent	Constituent	Color	Histological zone
Phloroglucinol + HCl	Lignin	Pink	Vascular bundles
Aniline sulphate + H_2SO_4	Lignin	Yellow	Vascular bundles
Weak Iodine solution	Starch	Blue	Vascular bundles, lamina
Ferric $chloride_{(aq.)}$	Tannins	Black	Vascular bundles
Dragendorff's reagent	Alkaloids	Light orange	Lamina
Libermann-Burchardt reagent	Steroids	Greenish	Lamina

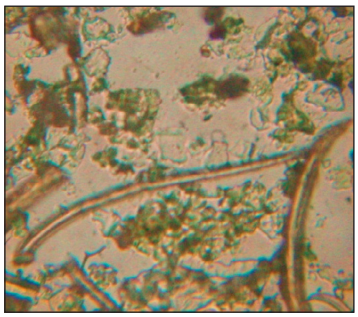
Table 5: Extraction value of ethnolic extract of powdered leaf of *Cissampelos pareira*

Sr. No.	Extract	Yield (%w/w)
1.	Ethanolic Extract	21.32

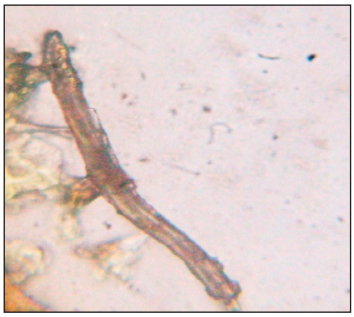
Table 6: Qualitative phytochemical analysis of ethanolic extracts of powdered leaf of *Cissampelos pareira*

Plant constituents	Ethanolic extract
Alkaloids	+
Saponins	+
Glycosides	+
Carbohydrates	+
Tannins and Phenolic Compounds	+
Flavonoids	+
Steroids	+
Proteins and Amino acids	+
Triterpenoids	–
Fixed Oils and Fats	–
Gums and Mucilage	–
Lignins	+

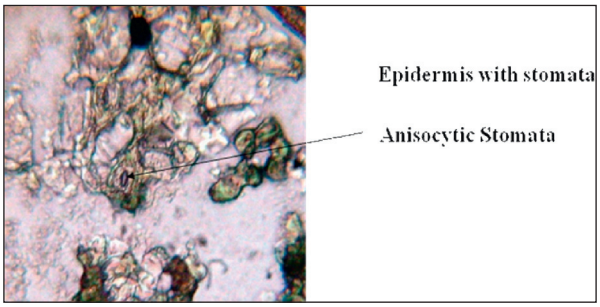
+: Present, –: Absent



a) trichome



b) Fibre



c) Epidermis with stomata

Xylem Fiber: Lignified

Covering Trichomes: unicellular, thick warty walls, acute apex, bulbous base.

Figure 3: Powder characteristics of *Cissampelos pareira Var. hirsuta*

DISCUSSION

In the last two decades of the century the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. The pharmacognostical study is one of the major criteria for identification of plant drugs. The present study on pharmacognostical characteristics of **Leaves of *Cissampelos Pareira*** will provide useful information for its correct identity. Studies on physicochemical constants and phytochemical screening can serve as a valuable source of information and provide suitable standards to determine the quality of this plant material in future investigations or applications.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Minoo H. Parabia, Botanist, Department of Bioscience, Veer Narmad South Gujarat University for authentication of plant sample.

REFERENCES

1. Yoganarasimhan S.N. *Medicinal Plants of India*, New Delhi: Interline publishing pvt. Ltd; 2002.
2. C.J. Saldanha C.J. *Flora of Karnataka*, New Delhi: Oxford and IBH publishing co; 1984.
3. Irama Ramírez, Alfredo Carabot, Pablo Meléndez, Juan Carmona, Manuel Jimenez, Asmita V Patel, Trevor A Crabb. Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*, Phytochemistry; 2003, 64:645-47.
4. Alain Fournet, Adrien Cavé, Pascale Duté, Jean-Frédéric Weber and Jean Bruneton. *Bis*-benzylisoquinoline alkaloids from *Abuta pahni*. Phytochemistry; 1987, 26:2136-2137.
5. Dewan S. Bhakuni, Sudha Jain and Rekha Chaturvedi. The biosynthesis of the alkaloids of *cissampelos pareira* linn. Tetrahedron; 1987:3975-3982.
6. Kupchan S.M. Patel A.C. and Fujita E. Tumor inhibitors VI. Cissamperine, new cytotoxic alkaloid from *Cissampelos pareira* Var. hirsuta. Cytotoxicity of Bisbenzylisoquinoline alkaloids. *JPharm Sci.* 1965 4:580-83.
7. Rojanasonthorn G. The isolation and characterization of bisbenzylisoquinoline alkaloid "tetrandrine" from the root of *Cissampelos pareira* Var. hirsuta l. [dissertation]. Bangkok, Thailand: Mahidol university; 1970.
8. Morita H., Matsumoto K., Takeya K., Itokawa H. and Iitaka Y. Structures and solid state tautomeric forms of two novel antileukemic tropoloisoquinoline alkaloids, pareirubrines A and B, from *Cissampelos pareira*. *Chem Pharm Bull(Tokyo)*; 1993, 8:1418-22.
9. Gordon A. Morris, Jonathan Castile, Alan Smith, Gary G. Adams and Stephen E. Harding. The effect of prolonged storage at different temperatures on the particle size distribution of tripolyphosphate (TPP) – chitosan nanoparticles. Carbohydrate polymers; 2004:399-400.
10. B. Vardhanabhuti, and S. Ikeda. Isolation and characterization of hydrocolloids from monoi (*Cissampelos pareira*) leaves. Food hydrocolloids; 2006:885-891.
11. Perez C. and Anesini C.. *In vitro* antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. *J Ethnopharmacol*;1994,1: 41-46.
12. Gessler M.C., Nkunyak M.H., Mwasumbi L.B., Heinrich M. and. Tanner M. Screening Tanzanian medicinal plants for antimalarial activity. *Acta Trop;*1994:65-77.
13. Caceres A., Giron L.M. and Martinez A.M..Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. *J Ethnopharmacol*; 1987:19(3):233-45.
14. Tripathi S.N, Tiwari C.M, Upadhyay B.N and Singh R.S. Screening of hypoglycemic action in certain indigenous drugs. *J Res Indian Med Yoga Homeopathy;* 1979:14(3):159-69.
15. Adesina S.K. Studies on some plants used as anticonvulsants in Amerindian and African traditional medicine. *Fitoterapia*; 1982(53):147-62.
16. Singh. K. K. and. Maheshwari J.K. Traditional phytotherapy of some medicinal plants used by the tharus of the Nainital district, UttarPradesh, India. *Int J Pharmacog*; 1994:32(1):51-58.
17. Caceres A., Cano O., Samayoa B. and Aguilar L..Plants used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against enterobacteria. *J Ethnopharmacol*; 1990:30(1):55-73.
18. Paulo Artur Konzen Xavier de Mello e Silva, Sídia Callegari-Jacques, Maria Helena Bodanese-Zanettini, Johansen D.A. Induction and identification of polyploids in *Cattleya intermedia* Lindl. (orchidaceae) by in vitro techniques. Cience. Rural; 2000:30.
19. Anonymous, *The Indian Pharmacopoeia*, New Delhi: Govt. of India publication; 1966, 947-950.
20. Singleton V.L., Rossi Colorimetry J.A. of total phenolic with phasphomolybdic – phosphotungstic acid agents.*Am. J. Enol.* vitic; 1965 16:144-153.
21. Houghton P.J., Raman A.. Laboratory Handbook for the Fractionation of Natural Extracts, Chapman and Hall, London; 1998:154-162.
22. Peach K., Tracy M.V. Modern Methods of Plant Analysis, Springer-Verlag, Heidelberg; 1955:3-4.

Pharmacognostic and Physicochemical study of *Punica granatum* L. leaf

Manisha Bapodara, Krunal Nagani and Sumitra Chanda*

Saurashtra University, Rajkot 360 005, Gujarat, India.

ABSTRACT

Punica granatum L. (Punicaceae) is a shrub, usually with multiple stems, that commonly grows 1.8-4.6m tall. The deciduous leaves are shiny and about 1-5 cm long. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. Hence, the present work was undertaken to establish the requisite pharmacognostic standards for evaluating the plant material. The present study includes examination of macroscopic and microscopic characters, powder analysis and physiochemical properties of *Punica granatum* L. leaf. The macro and microscopical studies indicated presence of simple leaf, opposite arrangement and prism and cluster crystals of calcium oxalate present throughout the transverse section. Chemomicroscopic characters present included starch and calcium oxalate crystals. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Key words: *Punica granatum* L., chemomicroscopic, pharmacognostic, physicochemical, crystals

INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the world for thousand of years and continue to provide mankind with new remedies. India is represented by rich culture, traditional and natural biodiversity offers a unique opportunity for drug discovery researchers.[1] According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[2,3]

Pharmacognosy has multidisciplinary characters i.e. to identify the drugs, its origin, morphology and microscopic studies, to determine the quality of the drug, its chemical compositions, therapeutic effects, etc.[4] Pharmacognosy studies help in identification and authentication of the plant material. The process of standardization can be achieved by stepwise pharmacognostic studies.[5] The standardization of a crude drug is integral part of establishing its correct identity. Before any crude drug can be in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established.[6] Therapeutic efficacies of medicinal plants depend upon the quality and quantity of chemical constituents. It has been established that chemical constituents of a plant species vary with regard to climate and seasons.[7]

Punica granatum L. (Punicaceae) is a small tree with potential human health benefits, is grown mainly in Iran, India, China, Japan, Russia and USA as well as in most near and far east countries. The pericarp of pomegranate as well as its roots, bark and juice are used in the treatment of colic, colitis-diarrhia, dysentery, leucorrhea, menorrhagia, oxyariasis, paralysis, rectocele and headaches in traditional medicine.[8] The different parts of the tree (leaves, fruits, flowers and bark skin) have been used traditionally for their medicinal properties. The fruit is reported for antioxidant.[9] and cancer prevention.[10] The stem is reported for antibacterial activity.[11] Nair and Chanda.[12,13] reported anticandidal activity and antibacterial activity of *P. granatum* leaf. Hepatoprotective role of flowers of *P. granatum* has been reported by Celik et al.[14]

The aim of the present study was to evaluate various pharmacognostic parameters like macroscopic and microscopic characters, powder characteristics and physicochemical properties of *Punica granatum* L. leaf.

***Address for correspondence:**
E-mail: svchanda@gmail.com

DOI: 10.5530/pj.2011.21.5

MATERIAL AND METHODS

Collection and extraction of plant material

The fresh leaf of *Punica granatum L.* was collected from Jamjodhpur, Gujarat in the month of August 2009. The plant was compared with voucher specimen (voucher specimen No. PSN311) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaf was washed under tap water, air dried, homogenized to fine powder and stored in airtight bottles. Ten grams of dried powder was extracted by sequential method using different solvents. The solvent was evaporated to dryness and the dried crude extract was stored in air tight bottle at 4°C. The acetone extract was used for the solubility study.

Pharmacognostic studies

Macroscopic characteristics

For morphological observations, fresh leaves (approx. 2-3 cm in length) were used. The macromorphological features of the plant parts (leaf) were observed under magnifying lens.[15]

Microscopic characteristics

Free hand section of leaf was taken and stained by well known reagent like safranine to confirm its lignification. Powder microscopy was also carried out and their specific diagnostic characters were drawn and recorded separately.[16]

Physicochemical parameters

Determination of physicochemical parameters as per guidelines of WHO[2] were also performed like total ash value, loss on drying, water soluble ash, acid insoluble ash, solubility, pH analysis, petroleum ether, acetone, methanol and water soluble extractive values, etc.

RESULTS AND DISCUSSION

Macroscopic characteristics

Macroscopically, the leaf was simple in composition, opposite, decussate, oblong-lanceolate or oblong-elliptic, glabrous, subsessile and exstipulate.The average leaf size was 2 to 3 cm (length) and 1 cm (width). The fresh leaf was green in color (Figure 1).

Microscopic characteristics

The transverse section of leaf of *Punica granatum L.* showed presence of upper and lower epidermis. The anomocytic and anisocytic stomata were present in epidermis. Unicellular trichomes were present in lamina but less in number. Xylem was lignified, phloem was non lignified, vascular bundles were arc shaped. Prismatic and cluster type of crystals of calcium oxalate were found. Spiral and annular types of xylem vessels were found in transverse section of leaf (Figures 2, 3).

The salient diagnostic characteristics of leaf were arc shaped vascular bundle, animocytic and anisocytic stomata, reticulate, annular and spiral types of xylem vessels and prism type of calcium oxalate crystal. These characters can be used for standardization of drugs and also used for preparation of plant monographs. Similar study is reported in other plants like *Manilkara hexandra,*[3] *Mitracarpus scaber* Zucc,[6] *Polyalthia longifolia* var. pendula,[17] *Tricosanthes cucumerina* L.,[18] *Ricinus communis* L.,[19] *Ficus racemosa* Linn.,[20] *Anisomeles indica* Linn.,[21]

Powder study

The crude powder of *Punica granatum* L. leaf was light green in color with characteristic odour and astringent test. The

Figure 1: Macroscopic characteristic of *Punica granatum* L. leaf

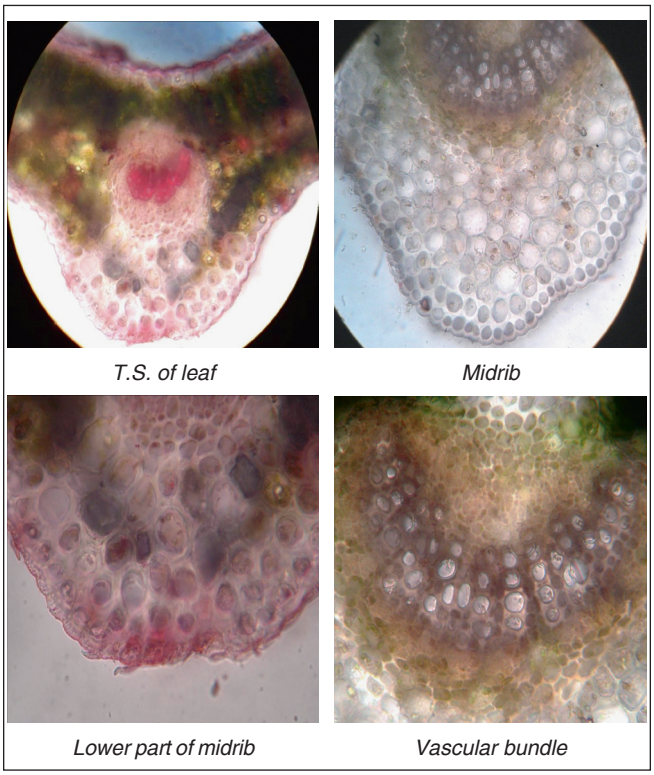


Figure 2: Microscopic characteristic of *Punica granatum* L. leaf

diagnostic features of powder were prism and cluster types of crystals of calcium oxalate present on surface of epithelial cells; presence of simple and compound starch grains. In surface view, fragments of epidermis were embedded with anisocytic and anomocytic stomata. Xylem vessels with annular, spiral and reticulated thickening was observed (Figures 4, 5).

Physiochemical properties

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of dry powder of leaves of *P. granatum* L. was 8.3% which is not very high, hence it would discourage bacteria, fungi or yeast growth. The ash value was determined by three different forms viz., total ash, acid insoluble ash and water soluble ash. The total ash is particularly important in the evaluation of purity of drugs; i.e. the presence or absence of foreign inorganic matter such as metallic salts or silica. Acid insoluble ash measures the amount of silica present, especially as sand. Water soluble ash is the water soluble portion of the total ash.[17] The total ash of crude powder of leaf of *P. granatum L.* was 7.6%, acid insoluble ash was 6.5% and water soluble ash was 6%. Less amounts of these three parameters show that the inorganic matter and silica was less in leaf of

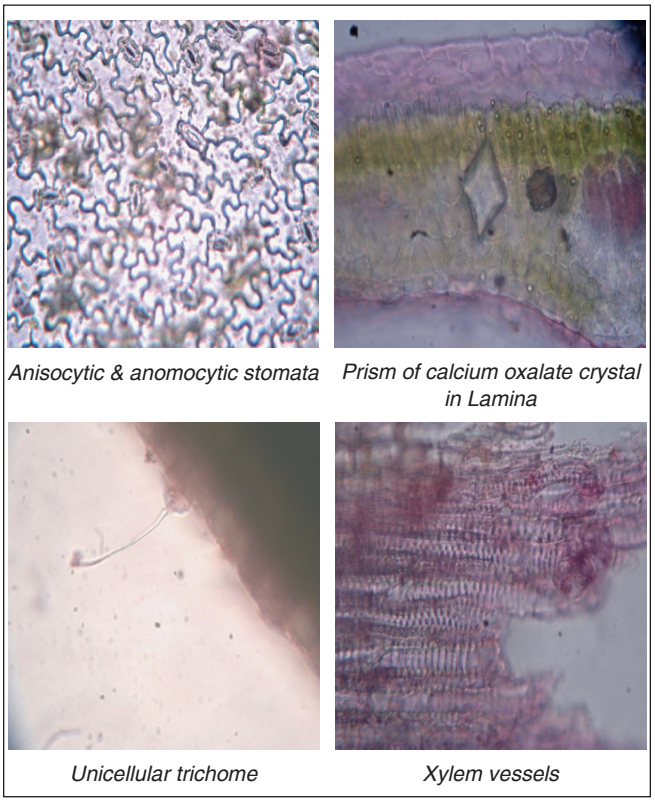


Figure 3: Microscopic characteristic of *Punica granatum* L. leaf

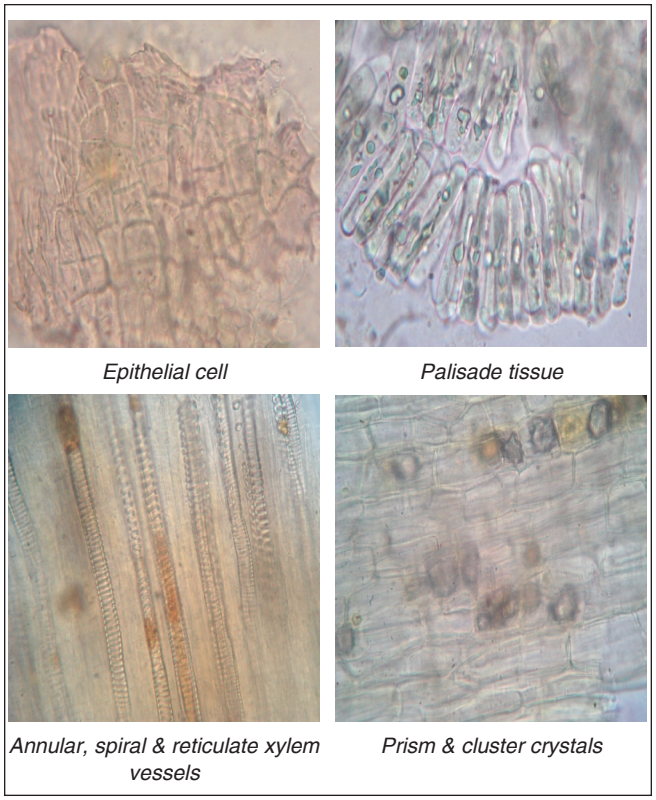


Figure 4: Powder study of *Punica granatum* L. leaf

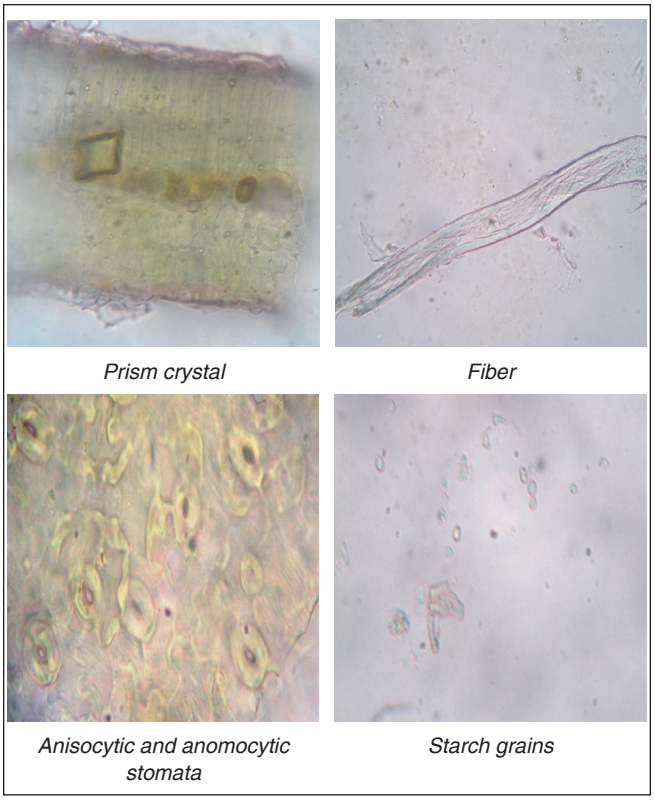


Figure 5: Powder study of *Punica granatum* L. leaf

Table 1: Proximate parameters of *Punica granatum* L. leaf

Parameters	Value % (w/w)
Loss on drying	8.3%
Total Ash	7.6%
Acid insoluble ash	6.5%
Water soluble ash	6%
Petroleum ether soluble extractive	1.46%
Acetone soluble extractive	6.23%
Methanol soluble extractive	27.76%
Water soluble extractive	31.09%
pH	3.45

Table 2: Solubility of acetone extract of *Punica granatum* L. leaf

Solvents	Solubility (mg/ml)
Petroleum ether	–
Acetone	17
Methanol	232.3
Ethyl acetate	0.7
Toluene	–
DMSO	34.7
DMF	295.9
D/W	12.2

P. granatum L. The extractive value of crude powder was maximum in water (31.09%) and minimum was in petroleum ether (1.46%). pH of acetone extract was 3.45 (Table.1).

Solubility test

The acetone extract of *P. granatum L.* leaf was evaluated for solubility in 8 solvents with varied polarities. The extract was highly soluble in methanol, dimethylformamide but insoluble in petroleum ether and toluene solvents (Table.2).

CONCLUSIONS

The microscopic characters, the physiochemical studies can be used for the quality control of the crude drug. Such a pharmacognostic study is useful for standardizing crude drugs and can be used to differentiate closely related species. This could also serve in the establishing data for preparation of monograph of this plant. Various physicochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug.

REFERENCES

1. Jachak SM, Saklani A. Challenges and opportunities discovery from plants. Curr Sci 2007; 92:1251-57.
2. Quality Control Methods for Medicinal Plant Materials (An authorized publication of World *Health Organisation, Geneva*). New Delhi: A.I.T.B.S. Publishers and Distributors (Regd.); 2002.
3. Chanda S, Nagani K, Parekh J. Assessment of quality of *Manilkara hexandra* (Roxb.) Dubard leaf (Sapotaceae): pharmacognostical and physicochemical profile. Phcog J. 2010; 2:520-4.
4. Jarald EE, Jarald SE. Textbook of Pharmacognosy and Phytochemistry. 1st edition CBS Publishers & Distributers, New Delhi. 2007
5. Ozarkar KR. Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* Linn. and *Valeriana wallichi* DC using mouse model. Ph.D. Thesis, University of Mumbai, Mumbai 2005.
6. Abere TA, Onwukaeme DN, Eboka CJ. Pharmacognostic evaluation of leaves of *Mitracarpus scaber* Zucc (Rubiaceae). Tropic J Pharm Res 2007; 6:849-53.
7. Mallavarapu GR, Ramesh S, Chandrasekaran RS, Rao RBR, Kaul PN, Bhattacharya AK. Investigation of the essential oil of Cinnamomum leaf grown at Bangalore and Hyderabad. Flavour and Frag J 1995; 10:231-49.
8. Schubert SY, Lansky EP, Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. J Ethnopharmacol 1999; 66:11-7.
9. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphiinidin, cyanidin, and pelargonidin. J Agric Food Chem 2002; 50:166-71.
10. Mehta R, Lansky EP. Breast cancer chemopreventive properties of pomegranate (*Punica granatum*) fruit extracts in a mouse mammary organ culture. Eur J Cancer Prev 2004; 13:345-8.
11. Nair R, Vaghasiya Y, Godvani N, Solanki A, Baluja S, Chanda S. Antibacterial activity of *Punica granatum* stem. Plant Arch. 2008; 8:671-3.
12. Nair R, Chanda SV. Anticandidal activity of *Punica granatum* exhibited in different solvents. Pharm Biol 2005; 43:21-5.
13. Nair R, Chanda SV. Antibacterial activity of *Punica granatum* exhibited in different solvents. Ind J Pharm Sci 2005; 67:239-43.
14. Ismail C, Atilla T, Ismail I. Hepatoprotective role and antioxidant capacity of pomegranate (*Punica granatum*) flowers infusion against trichloroacetic-exposed in rats. **Journal name missing** 2009; 47:145-9.
15. Khandelwala KR, Kokat CK, Pawar AP, Gokhale SB. Practical Pharmacognosy. 2nd Ed. Nirali Prakashan. 1988; 31-40.
16. Johansen DA. Plant Microtechnique, 1st Ed. Mc Graw Hill Book Co., Inc. New York, London. 1940.
17. Dave R, Nagani K, Chanda S. Pharmacognostic studies and physicochemical properties of the *Polyalthia longifolia* var. pendula leaf. Phcog J 2010; 2:572-76.
18. Sandhya S, Chandrasekhar J, David Banji, Rao KNV. Pharmacognostical study on the leaf of *Tricosanthes Cucumerina* Linn. Arch Appl Sci Res 2010; 2:414-21.
19. Singh RK, Gupta M K, Singh AK, Sunil Kumar. Pharmacognostical investigation of *Ricinus communis* stem. Int J Pharma Sci Res 2010; 6:89-4.
20. Gorwadiya HC, Savalia RM, Vachhani KV, Desai TR, Pandya DJ. Pharmacognostic study and establishment of quality parameters of leaves of *Ficus racemosa* Linn. Phcog J **year missing** 2:15-20.
21. Ushir YV, Chidrawar VR, Patel KN, Tatiya AU, Surana SJ. Pharmacognostic studies on the leaves of *Anisomeles indica* Linn. (Labiatae). Phcog J. 2009; 1:44-8.

Pharmacognostic and Phytochemical investigations of aerial parts of *Acalypha indica* Linn.

Takle V.V.*[1], Savad R.V.[1] KandalkarA.M.[2], Akarte A.M.[2], Patel A.M.[3]

[1]*Department of Pharmacognosy and Phytochemistry, K.L.E's College of Pharmacy, Vidyanagar, Hubli-580031, Karnataka, India.*
[2]*Institute of Pharmaceutical Education, Boradi, Tal-Shirpur Dist. Dhule, 425428, Maharashtra, India.*
[3]*Sant Gajanan Maharaj Rural Pharmacy college, Mahagaon Tal- Gadhinglaj Dist. Kolhapur, 416503, Maharashtra, India.*

ABSTRACT

In the present study, aerial parts of *Acalypha indica* Linn. belonging to family Euphorbiaceae was subjected to basic pharmcognostic examinations like morphological, and histological evaluations, Quantitative Microscopy including ash values, extractive values and preliminary phytochemical screening. The plant material after defatting with pet. Ether (60-80) extracted with ethanol and fractionated with chloroform, ethyl acetate and n-butanol. Qualitative chemical tests of various extracts and fractions revealed the presence of Alkaloids, Steroids, Flavonoids, Glycosides, Tannins, Carbohydrates, etc. These observations will enable to standardize the botanical identification of the drug in its crude form. Data obtained in this investigation can be used as standards of pharmacopoeial guidelines in inclusion of this drug studies of herbal monograph

Key words: *Acalypha indica*, Pharmacognostic, morphological, histological, Phytochemical.

INTRODUCTION

Today 80% of world population is depends on the herbal medicine as a primary as a primary health care, with the recent advances in the field of pharmacy it is now some what easy to obtained the data of the medicinal plant and to give the scientific back ground to the traditional claims. *Acalypha indica* is an erect annual herb of 30-100 cm in height occurring as a weed throughout the plains of India, hills of Orissa up to 210 m. it also found in hottest parts throughout the world.[1] It has so many therapeutic actions such as laxative, anti-bacterial, anthelmintic, anti-diabetic, expectorant, etc.[2,3] The plant is reported to have a post-coital antifertility effect.[4] The plant is reported to have antivenum properties.[5] Acalypha indica is also having diuretic effect.[6]

Now a days it is very essential to obtain the pharmacognostic and phytochemical data of the various medicinal plant in order to give the scientific background to the traditional claims. The present study was under taken to develop pharmacognostical parameters such as morphology, proximate values and phytochemical studies of aerial parts of *Acalypha indica* Linn.

***Address for correspondence:**
E-mail: cognosist@rediffmail.com

DOI: 10.5530/pj.2011.21.6

MATERIALS AND METHODS

Collection and authentication

The aerial parts of *Acalypha indica* linn. were collected from the local areas of Hubli-Dharwad in the month of july[3,4] and authenticated by Dr. B.D.Huddar, Head of the Department of Botany, Shri Kadasiddheshwar H.S. Kothambari Science institute, Vidyanagar, Hubli, India. A voucher specimen of 08PG-657 is preserved at KLES's college of pharmacy, Hubli, India

Pharmacognostical Studies

The morphological prominent fetchers like color, odor, taste, size, shape, apex, margin, base, venation pattern, petioles, surface including composition of the Leaves were studied and documented according to the standard Procedures.[5,6] The Transverse section of leaf & stem (Figure 1) was taken and developed with phloroglucinol and HCl.[7,8] The powder drug studies are carried out with saffranin, sudan red, phloroglucinol and HCl, for different diagnostic characters[9] (Figure 2). The Quantitative Microscopy[7] (Figure 3) and the other proximate values such as ash value, extractive values, and moisture content was carried out by standard procedure.[10,11]

Phytochemical investigations

The coarse powder of shade dried aerial parts of *A. indica* was defatted with pet. ether (60-80°C) and then extracted

with ethanol.[12,13] The ethanolic extract was further fractionated with chloroform, ethyl acetate, and finally with n-butanol according to increasing order of their polarity.[14] The extracts and fractions were concentrated, dried and subjected for phytochemical investigations by qualitative chemical tests to detect the presence of various phytoconstituents.[15] (Table.2)

RESULTS AND DISCUSSION

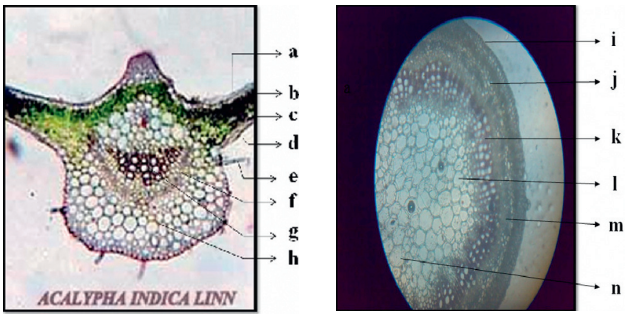
a. Morphological Evaluation

As per the observations the *Acalypha indica* collected from the region of Hubli-Dharwad is an erect annual herb, 30-60 cm in height. Leaves are dark green on dorsal side and light green on ventral side with smooth texture and serrate-crenate margin. They are ovate or rhombic-ovate, 3-6 cm. long and 3-4 cm broad with slender petioles which may be longer than the blades. It has Characteristic and admissible odor; male flowers minute, clustered at the top, and female flowers with accrescent, broad, leafy bract; capsules often one-seeded, concealed in the bract; seeds pale-brown, ovoid, acute, smooth.

b. Microscopical Evaluation

T.S. of *Acalypha indica* leaf (Figure 1) shows the presence of Upper and Lower Epidermis containing single layered cells covered with thick cutical. Then mesophyll is differentiated in to palisade and spongy Parenchyma. Palisade Parenchyma is one layered, compa ct with radially elongated cells whereas spongy Parenchyma is many layered, loosely arranged with intercellular spaces. The collenchyma consists of thick walled collenchymattous cells below the vascular bundle. Vascular bundle shows xylem towards upper epidermis and phloem towards lower epidermis. In between xylem cell, xylem parenchyma is found. The phloem is found below xylem. Trichomes are multicellular epidermal hairs present on upper epidermis (very rare) and lower epidermis (numerous).

Stem (Figure 1) in transection circular in outline and shows epidermis single layered with Few layers of polygonal tabular cells, alternate layer of thick and thin walled cork cells. The Several layers of thin walled tangentially elongated cells containing reddish brown matter show the cortex layer. Xylem fibres are well developed, brownish red in colour, consist of vessels and parenchyma, where as Phloem fibres are Thick walled, cellulosic in the inner part and lignified in the outer part. Pith contains Parenchymatus cells with intercellular spaces. Pith is absent in the root cells.



Leaf: *a) Upper Epidermis; b) Palisade tissue; c) Spongy tissue; d) Upper Epidermis; e) Trichomes; f) Xylem; g) Phloem; h) Collenchyma*

Stem: *i) Cork; j) Cortex; k) Xylem fibres; l) Collenchyma; m) Phloem fibres; n) Pith*

Figure 1: T.S. of leaf and Stem of *A. indica* Linn.

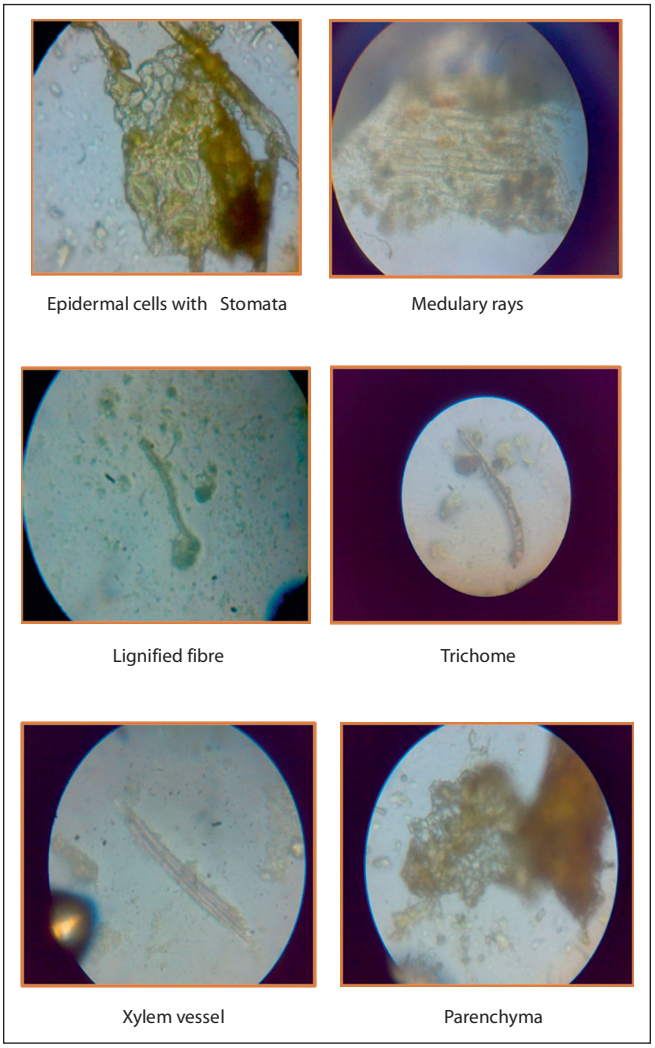


Figure 2: Powder Microscopy of *A. indica* Linn.

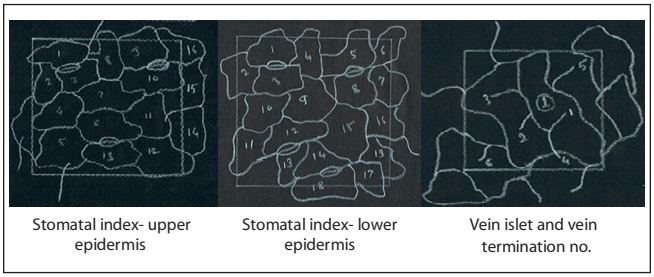


Figure 3: Quantitative Microscopy of *A. indica* Linn.

Table 1: Proximate values

Ash values				Extractive values			Moisture content
Total ash	Sulphated ash	Water soluble ash	Acid insoluble ash	Alcohol	Water	Pet. Ether	
16%	26%	2.33%	0.58%	7%	20%	3%	09.50%

Table 2: Preliminary phytochemical investigations

Phytoconstituents	Petroleum ether (60-80°C)	Ethanol extract	Fractions		
			Chloroform	Ethyl acetate	n-Butanol
Steroids	+	+	–	–	–
Triterpenoids	+	+	–	–	–
Glycosides	–	+	+	–	–
Carbohydrates	–	+	–	–	–
Alkaloids	–	+	+	–	–
Flavonoids	–	+	–	+	+
Tannins	–	+	–	+	+

"+" Present, "–" Absent

Quantitative Microscopy

Quantitative microscopy of leave of Acalypha *indica* revealed for stomatal index, Vein islet number and Vein termination number (Figure 3) which are very specific character for identification and characterization of leaf crude drug. In this study the stomatal index for upper and lower surface was found to be 15-16 and 17-18 respectively. Whereas Vein islet and vein termination number was found to be 2-3 and 16-17 respectively.

Powder microscopy

The powder characteristics of dried powder of *Acalypha indica* (Figure 2) show the presence of Fibers which are large, lignified with moderately thickened walls. Epidermal cells with paracytic stomata. Calcium oxalate crystals are square in shape. Xylems vessels are numerous, bordered, thickened, and frequently associated with other xylem elements. Trichomes are larg in numbers; Medullary rays are parenchymatous & multiseriate.

c. Physico-Chemical Values

Various Physical Values namely Extractive value, Moisture content, and Ash values was mentioned in Table 1.

d. Phytochemical investigations

Qualitative chemical examinations of various extracts and fractions revealed the presence of Alkaloids, Steroids, Flavonoids, Glycosides, Tannins, and Carbohydrates. Results are expressed in Table 2.

CONCLUSION

From the pharmacognostic, and phytochemical investigations, it is quite possible to set the standards of this plant as per the pharmacopoeial guidelines and further additional contribution like isolation of phytoconstituents is required for the fulfillment of the studies regarding Herbal monograph.

REFERENCES

1. The wealth of India, Raw materials Council of scientific and Industrial Research. (Vol I). New Delhi-India, Reprinted-P.47-48. 2003.
2. Kirtikar KR, and Basu BD. "Indian Medicinal Plants" (Vol III). 2nd ed. Dehradun: International books distributers; p.2262-64 1995.
3. Yogganarasimhan SN. "Medicinal Plants of India". 1st ed. Bangalore: Interline Publishing Pvt. Ltd.; p.9-10 1996.
4. Bedon E,Hatfield GM. An investigation of the antiviral activites of *Phodophyllum peltun.* Lloydia.1982;45(6):725.
5. Annie S, Rajendran k, Ramgopal B, Denish Kumar C. Neutralization potential of vivper russelli (Russell's viper) venom by ethnol leaf extract of *Acalypha indica.* J. Enthopharmaccol. 2004; 94:267-73.
6. Das AK, Ahmed F, Biswas NN, Dev S, Masud MM. Diuretic Activity of *Acalypha indica*. Dhaka Univ J Pharm Sci. 2005; 4:1-2.
7. Nandhakumar M, Tamil Iniyan G, Senthil kumar M, Dinesh Kumar B, Mitra A. In Vitro Assay of Alpha Amylase Inhibitory Activity of Indian Medicinal Herb *Acalypha Indica*. Journal of Clinical and Diagnostic Research. April; 3:1475-78 2009.
8. Mukherjee PK, Quality Control of Herbal Drugs – (2002)An Approach to Evaluation of Botanicals,Business Horizons Pharmaceutical Publishers, 1st ed. p.138-141.
9. Wallis TE."Textbook of Pharmacognosy"5th ed. New Delhi: CBS Publishers and Distributors; p.567-636 1985.
10. Khandelwal KR. "Practical Pharmacognosy". 18th ed. Pune: Nirali prakashan; p.15-19 2007.
11. Kokate CK. "Practical Pharmacognosy". 17th ed. Pune: Nirali Prakashan; p.1-13 1999.
12. Iyengar MA. "Pharmacognosy of powdered crude drugs". 1st ed. Manipal: Iyengar Publications; P.1-7, 21-31, 57-60 1980.
13. "Quality Control Methods for Medicinal Plant materials". 1st ed. New Delhi: AITBS publisher and distributors; p.10-45. 2002.
14. "Indian Pharmacopoeia". Vol-II: New Delhi: Published by the controller of publication; A-3, 45, 53-55 1996.
15. Atta AH, Mohamed NH, Nasr SM, Mounair SMPhytochemical & pharmacological studies on *Convolvulus fatmensis* Ktze. Journal of natural remedies; 7(1):109-19 2007.
16. Houghton PJ, Raman A. "Laboratory handbook for the fractionation of natural extracts". 1st ed. London: Chapman and Hall; p.22-56,146-50 1998.
17. Solomon Habtemariam a, Jackson C. Antioxidant and cytoprotective activity of leaves of *Peltiphyllum peltatum* (Torr.) Engl. Food Chemistry; 105:498-503 2007.
18. Yusha'u M, Aliyu BS, Olonitola SO. Preliminary Screening of *Acalypha* Extracts for *In Vitro* Inhibitory Activity against Extended-Spectrum B-Lactamase Producing Enterobacteriaceae. International journal of pure and applied sciences; 2(2):1-5 2008.

Cytotoxicity and Antioxidant Activity of New Biologically Active Constituents from *Salvia Lanigra* and *Salvia Splendens*

Usama Y. Shaheen[1]*, Mohammed H. Hussain[1] and Hassan A. Ammar[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. [2] Department of Pharmacognosy, Faculty of Pharmacy, Ahram Canadian University in Egypt, 6th October, Egypt.

ABSTRACT

Chromatographic fractionation of the acetone extracts of each of *Salvia lanigra* and *Salvia splendens* and of the n-butanol extract of *Salvia lanigra*, resulted in the isolation and identification of two known diterpenes; horminone (1) and 7-*O*-ethylhorminone (2), three new diterpenes; salviatane B (3), salvianol A (4) and salviaclerodan A (5), two known triterpenioc acids; ursolic acid (6) and oleanolic acid (7), a known sterol; β-sitosterol (8), two known flavones; salvigenin (9) and apigenin (10) and one new caffiec acid dimmer; 3,3′-dehydrodicaffeic acid (11). The cytotoxicity and the antioxidant activity of the different extracts (MeOH, acetone and n-butanol) of both *Salvia lanigra* and *Salvia splendens* and most of the isolated pure compounds (1-5, 9 and 10) were determined.

Key words: Salvia, Diterpenes, Phenolics, Cytotoxicity and Antioxidants Activity.

INTRODUCTION

Salvia is an important genus consisting of ca 900 species in the family Lamiaceae (formerly Labiatae) and some species of *Salvia* have been cultivated worldwide for use in folk medicines and for culinary purposes.[1] The genus has attracted great interest so much so that it has been the subject of numerous chemical studies. It is a rich source of diterpenoids, tanshinones and polyphenols.[2-4] These compounds constitute the major secondary metabolites and show interesting spectra of biological activities as antioxidant, antitumor, cytoprotective, antibacterial and as components of herbal teas which has been used extensively for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrheal, flatulence, dyspepsia, gastritis, sore throat, tuberculosis, psoriasis, eczema and neuroasthenic insomnia.[1,5] In the flora of Egypt, the genus *Salvia* is represented by three major species; *S. lanigra* Poir, *S. splendens* Sello, and *S. farinacia* Benth. This study was undertaken to perform the isolation, structure elucidation of the interesting constituents of *S. lanigra* Poir and *S. splendens* Sello. Also to perform the screening of cytotoxic activity against certain human cell lines and the antioxidant properties using inhibition of DPPH• method of different extracts (MeOH, Acetone and *n*-butanol) as well as some isolated pure compounds (**1-5, 9** and **10**).

Address for correspondence:
E-mail: usamayosef2003@yahoo.com

DOI: 10.5530/pj.2011.21.7

MATERIAL AND METHODS

General Experimental Procedures: UV spectra were determined with a Hitachi 340 spectrophotometer; IR spectra were carried out on a Nicollet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The ^{1}H and ^{13}C NMR measurements were obtained with a Bruker NM spectrometer operating at 300, 400 and 500 MHz (for ^{1}H) and 100 and 75 MHz (for ^{13}C) in Acetone-d_6, DMSO-d_6 or $CDCl_3$ solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135°). HMQC NMR experiments were carried out using a Bruker AMX-500 high field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1 or NUTS program for NMR. HRFAB mass spectra were performed on a VGZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.). MALDI-TOFMS was conducted using perceptive Biosystems, Voyager DE-STR mass spectrometer. ESIMS (positive and

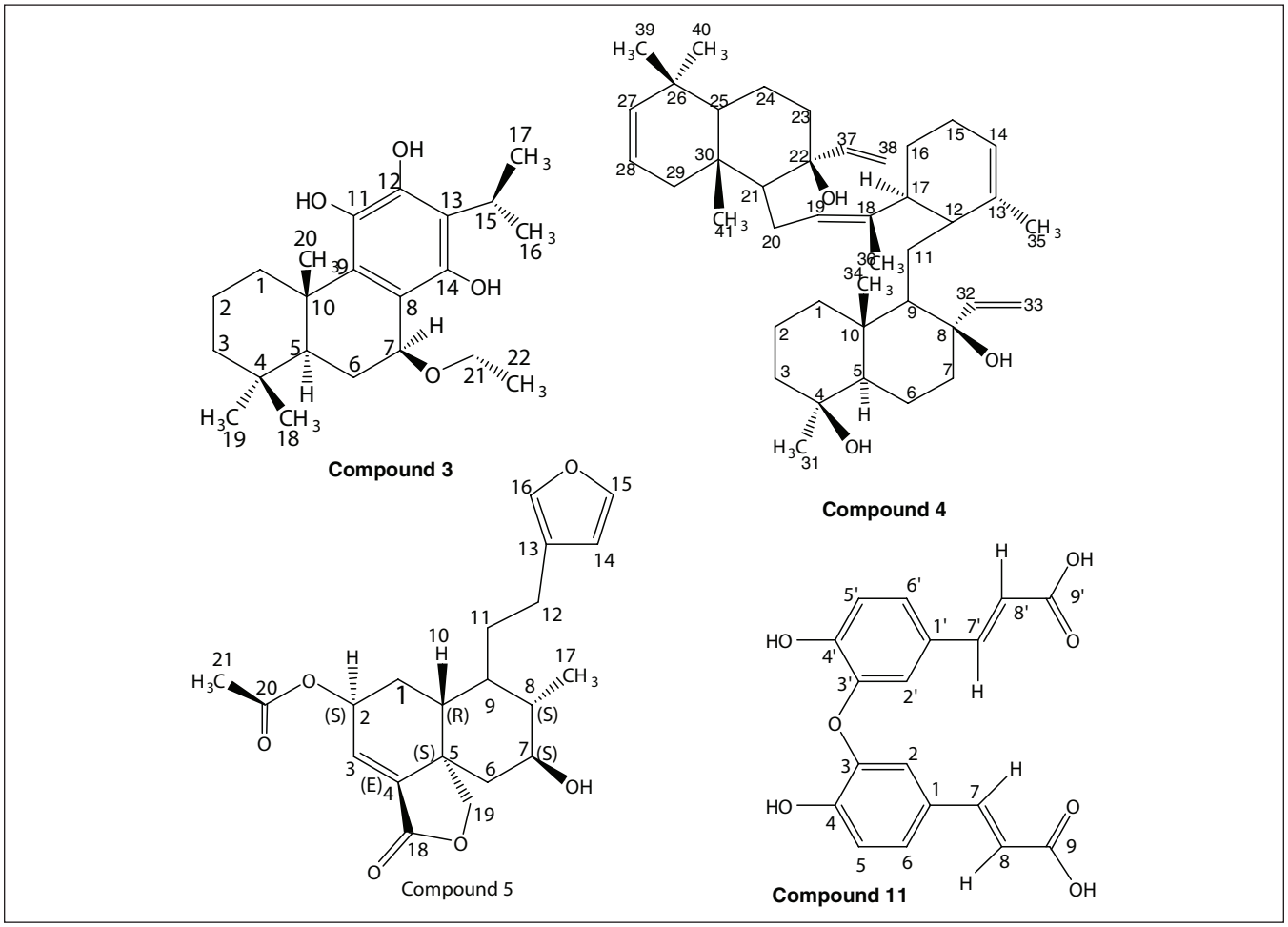


Figure 1: Structures of compounds 3-5 and 11.

negative ion acquisition mode) was carried out on a TSQ700 triple quadrupole instrument (Finnigan, SanJose, CA, USA) mass spectrometer. EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF,X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck), were used for open column chromatography. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 for 5 min and diazotized sulfanilic acid (Pauly's reagent) for phenols.

The following material and reagents were used for cell culture and cytotoxic assays. Human liver hepatocellular carcinoma (SNU-398 and Hep G2), Liver hepatoma (PLC/PRF/5), Kidney hypernephroma (SW 156), Kidney carcinoma (A-498), Urinary bladder carcinoma (HT-1376), Urinary bladder transitional cell carcinoma (UM-UC-3), Stomach gastric carcinoma (Hs 746T and Hs 740.T), ovary adenocarcinoma (NIH:OVCAR-3 and SK-OV-3), Lymph node Hodgkin's disease; Hodgkin's lymphoma (Hs 388.T and Hs 751.T) and Uterus Uterine sarcoma (MES-SA and MES-SA/MX2) cell lines were purchased from the American Type Culture Collection (ATTC). Dulbecco's Modified Eagle Medium (DMEM) was from (Gibco, Grand Island NY, USA). Eagle Minimum Essential Medium (EMEM) and Roswell Park Memorial Institute (RPMI) 1640 medium were from (Nissui Pharm. Co., Ltd., Tokyo, Japan). Flat-bottom plates, 96 well were from (Iwaki Glass Co., Ltd., Fumabashi-Chiba-Ken, Japan). 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), for colorimetric assay was from Sigma (St. Louis, Mo., USA). 10% Fetal Bovine serum (FBS) was from (Gibco Br L, Rockville, MD, USA). All other chemicals used were of analytical reagent grade. The following material and reagents were used for antioxidant activity. 2, 2- Diphenyl -1-picrylhydrazyl, 95 % was from (Aldrich, D 211400 -1G, USA). Automatic pipette was from (Gilson, France). 96-well plates were from (USA). Incubator (30 ± 2 °C) was from (New Brunswick Scientific, Innova 5000 Gyratory tier shaker, USA). Multi well scanning spectrophotometry was from (Dynex MR 5000, Chantilly, VA, USA). D,

L-α-tocopherol was from (Nacalai, Tesuque, Tokyo, Japan). Butylated hydroxytoluene (BHT) was from (Sigma, chemical company, St. Louis, Mo, USA).

DPPH stock solution; dissolve 125 mg of DPPH in 100 ml of reagent alcohol, Sonicate for 10 minutes. The stock solution may be stored for 2 weeks in the refrigerator (–40°C) under Argon. DPPH working solution; dilute the stock solution 1: 10 (V/V) in reagent alcohol. The concentration of DPPH in the working solution is 316 μM.

Sample stock solution; dissolve a known amount of sample (extracts, compounds and standards) in 10 ml of reagent alcohol. The concentration of the stock solution is given by such that the working solutions prepared from it will provide an even distribution of data points around the 50% inhibition point (EC_{50}). For many samples, several trials may have to be run to determine the stock solution concentration that provides an appropriate range of data points. Stock solutions; mixed tocopherols (1.25 mg /ml), BHT 1.25 mg /ml, crude methanolic, acetone and *n*-BuOH extracts of *Salvia lanigra* and *Salvia splendens* (5 mg/ml), and isolated compounds (1 mg/ml).

Plant Material: The plant materials used in this work consisted of the leaves and stems of *Salvia lanigra* Poir, collected in April, 2002, from Borg Al-Arab Desert, Alexandria, Egypt. The leaves and stems of *Salvia splendens* Sello collected in April, 2002 from Zoo garden Giza, Egypt. The two plants were kindly identified by Late Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University, and Engineer Badia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza.

***Extraction and Isolation*; *Salvia lanigra* Poir:** Three kg of air-dried powdered (leaves and stems) of *Salvia lanigra* were subjected to exhaustive extraction with acetone (3 x 12 L). The combined acetone extract was concentrated under *vacuo* at 40°C to dryness (140 g). The marc was then partitioned several times with *n*-butanol (3 x 3 L), the concentrated *n*-butanol extract was concentrated under *vacuo* at 40°C to dryness (14 g). The acetone extract was applied to silica gel column using n-hexane: EtOAc (100:00-70:30), to yield six fractions (A-F). Fr. A (4.7g) was further subjected to silica gel flash column chromatography using n-hexane: EtOAc (100:00-90:10) to give five fractions (A1-A5). Four fractions; Fr. A1 (195mg), Fr. A2 (180mg), Fr. A3 (560mg) and Fr. A4 (1.95g), were further subjected separately to a series of column chromatographic techniques including silica gel column (n-hexane: EtOAc- 95:5-85:15), silica gel flash column (Petroleum ether: EtOAc- 90:10-80:20), Sepralyte C_{18} flash column (H_2O: MeOH- 65:35-100:00) and final purification by Sephadex LH-20 column (MeOH) to afford compounds **1** (93mg), **2** (19 mg), **3** (23 mg) and **4** (35 mg), respectively. Fr. C (10.95 g) was chromatographed over silica gel flash column using n-hexane: EtOAc (90:10-80:20) to give three fractions of C1, C2 and C3. Fr. C1 (225mg) and Fr. C2 (6.8g) were further subjected separately to silica gel column using n-hexane: EtOAc (9550-85:15), Sepralyte C_{18} flash column (H_2O: MeOH- 50:50-90:10) and Sephadex LH-20 column (MeOH) to afford compounds **7** (3.7 g) and **9** (23 mg), respectively. The n-butanol fraction was subjected to silica gel column chromatography using $CHCl_3$: MeOH (100:00-70:30) to give six fractions (A-F). Fraction C (220 mg) and fraction E (354 mg) were further subjected separately to silica gel column using $CHCl_3$: MeOH (95:5-80:20), silica gel flash column using CH_2Cl_2: MeOH (90:10-75:25), Sepralyte C_{18} flash column (H_2O: MeOH-40:60-20:80) and Sephadex LH-20 column using MeOH and $CHCl_3$: MeOH (15:85) to afford compounds **10** (23 mg) and **11** (18 mg), respectively.

***Salvia splendens* Sello:** Two kg of air dried powdered (leaves and stems) of *Salvia splendens* was subjected to exhaustive extraction with acetone (3 x 8 L). The combined acetone extract was concentrated under *vacuo* at 40°C to dryness (76 g). The acetone extract was chromatographed over silica gel column using n-hexane: EtOAc (100:00-70:30), to give seven fractions (**A-G**). Fr. D (1.5 g) was subjected to silica gel flash column (Petroleum ether: EtOAc- 90:10-75:25) and Sepralyte C_{18} flash column (H_2O: MeOH- 60:40-85:15) to give two fractions of D1 and D2. Fr. D2 (455 mg) was further subjected to silica gel column using Petroleum ether: EtOAc (85:15-80:20) to afford compound **8** (205 mg). Fr. E (1.25 g) was subjected to silica gel column (Petroleum ether: EtOAc- 90:10-75:25), silica gel flash column using n-hexane: EtOAc (90:10-70:30) and Sepralyte C_{18} flash column (H_2O: MeOH- 70:30-90:10) to give two fractions of E1 and E2. Fr. E1 (50 mg) was further subjected to Sephadex LH-20 column (MeOH) to afford compound **5** (205mg), while Fr. E2 (50 mg) was further subjected to Sephadex LH-20 column (MeOH) to afford compound **6** (120 mg).

Cytotoxicity assay:[6,7] Cell lines SNU-398, Hep G2, PLC/PRF/5, A-498, HT-1376, UM-UC-3, Hs 746T, Hs 740.T, Hs 388.T, Hs 751.T, MES-SA and MES-SA/MX2 were cultured in Dulbecco's Modified Medium (DMEM) containing 10% heat-inactivated Fetal Bovine Serum (FBS). The SW 156, SK-OV-3, and NIH:OVCAR-3 cells were cultured in Eagle Minimum Essential Medium (EMEM) containing Earle's salts heated and sublimated with amino acids and 10% heat inactivated Fetal Bovine Serum (FBS). The SNU-398, A-498, UM-UC-3, MES-SA and MES-SA/MX2 cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 Medium containing 10% heat inactivated Fetal Bovine serum (FBS). All cell lines were

cultivated in an incubator at 37°C in humidified air containing 5% CO_2. For routine cytotoxicity assays, all cell lines were adapted to one single medium RBMI 1640 medium sublimated with 10% FBS, 2 mM L-glutamine, penicillin (100 unites/1 ml), and streptomycin (100 µg/1 ml). For the micro assay, the growth medium was supplemented with 10 mM HPES (1-[2-hydroxy ethyl] piprazine-4-ethane sulfonic acid) buffer pH 7.3 and incubated at 37°C in CO_2 incubator. Cellular viability in the presence and absence of experimental reagents was determined using the standard MTT (3-[4, 5-dimethyl thiazole-2-yl]-2, 5-diphenyl tetrazolium bromide) colorimetric assay. The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically. In brief, exponentially growing cells were harvested and 200 µL cells suspension were seeded in 96-well microplates and preincubated for 24 h at 37°C under 5% CO_2 to allow cell attachment. After attachment 10 µL of an EtOH: H_2O (1:1 solution) containing varying concentrations of test samples; different extracts (MeOH, Acetone and *n*-BuOH) and isolated compounds (**1- 6, 11-19**) were added in wells in duplicate, and 10 µL EtOH: H_2O (1:1) was added into wells as a control. Sample containing microplates were further incubated for 6 days. Cell survival was evaluated by adding 10 µL of 5 mg/ml MTT in 0.1 mM, pH 7.4 phosphate buffered saline to each well, and re-incubating plates in 5% CO_2 /air for 4 hrs at 37°C. Plates were then centrifuged at 1500 × *g* for 5 minutes to precipitate cells and MTT formazan. An aliquot of 100 µL of the supernatant was removed, and DMSO (100 µL) was added to dissolve precipitated, reduced MTT. The plate was mixed on a microshaker for 10 minutes, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (Dynex MR 5000) Chaltilly VA, USA). The ED_{50} value, which reduces the viable cell number, was defined as the concentration of test samples resulting in a 50% reduction of absorbance compared to untreated controls. The 50% effective dose (ED_{50}) obtained by measuring growth inhibition with MTT, are shown in (Tables 4 and 5).

Statistical Analysis:[8] All cytotoxic data were expressed as mean (± SE. Student's *t*-test was applied for detecting the significance of difference between each sample; $P < 0.05$ was taken as the level of significance.

Antioxidant activity assay:[9] DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 515 nm on a UV/visible light spectrophotometer. The reduction in absorbance at 517 nm can be measured to determine the antioxidant potential measured by comparing IC_{50} to known antioxidant such as tocopherols, Butylated hydroxytoluene (BHT).

In a 96-well plate, add 10 µL of sample working solution to 190 µL of DPPH working solution. Prepare a blank by adding 10 µL of ethanol to 190 µL of DPPH working solution. The final volume in each is therefore equal to 200 µL. The concentration of DPPH in final solution is approximately 300 µM. Incubate solutions to (30 ± 2°C) for 30 minutes. After incubation, measure the absorbance of each solution at 517 nm. Plot % inhibition vs. the concentration (mg/ml) of test sample in the final solution. From the equation of the line, calculate the test sample required to reduce the absorbance at 517 nm by 50% (IC_{50}).

Calculations:

$$\text{DPPH Scavenging Effect (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\right] \times 100$$

1. Plot % inhibition vs. the concentration (mg/ml) of test sample in the final solution.
2. From the equation of the line, calculate the test sample required to reduce the absorbance at 517 nm by 50% (IC_{50}).

The results of different extracts, isolated compounds as well as mixed tocopherols and BHT were shown in (Table 6).

RESULTS AND DISCUSSION

The air-dried leaves and stems of *Salvia lanigra* and *Salvia splendens* were subjected separately to exhaustive extraction with acetone. The acetone extracts were partitioned several times with *n*-butanol. The acetone extracts of *Salvia lanigra* and *Salvia splendens* and the *n*-butanol extract of *Salvia lanigra* were repeatedly chromatographed over normal-phase silica gel, silica gel for flash and reversed-phase silica gel to give five diterpenes (**1**-**5**), two triterpenioc acids (**6** and **7**), one sterol (**8**), two flavones (**9** and **10**) and one phenolic acid (**11**). The known compounds were identified as horminone (**1**)[10-12] and 7-*O*-ethylhorminone (**2**),[10-13] ursolic acid (**6**) and oleanolic acid (**7**),[14] β-sitosterol (**8**),[15,16] salvigenin (**9**) and apigenin (**10**) by comparing their spectral data (mass spectrometry, ^{1}H- and ^{13}C NMR) with those reported previously.[17-19]

Compound 3: was obtained as a yellowish amorphous solid. Its molecular formula $C_{22}H_{34}O_4$ was determined from an $(M + H)^+$ ion at m/z 363 in the positive ion ESI-MS. Other fragment ion at 317 $(M + H - CH_3CH_2O)^+$ indicated the presence of an ethyl moiety. Analysis of the IR spectrum of (**3**) suggested that it contained a hydroxyl (3420 cm^{-1}), and aromatic (1502, 1580, 1615 cm^{-1}) groups. The UV

spectrum of (**3**) showed absorption bands at λ_{max} 245 and 280 nm supporting the presence of an aromatic ring. ^{1}H and ^{13}C chemical shifts for (**3**) suggested an aromatic C-ring, substituted with an isopropyl group at C-13 and hydroxyl groups at C-11, C-12 and C-14 in the abietane diterpene skeleton.[10-12] In the ^{1}H NMR spectrum, signals for three tertiary methyl singlets were observed at δ 0.92 (3H), 0.93 (3H), 1.22 (3H) which were attributed to the geminal dimethyl groups (C-18 (*a*), C-19 (*β*)) and C-20, respectively, two secondary methyl signals at δ 1.23 and 1.18 (each 3H, d, J = 6.8 Hz, Me-16, 17).

The latter two signals, together with a benzylic methine signal at δ 3.18 (1H, heptet, J = 7.0 Hz, H-15), showed the presence of an isopropyl group, characteristic for all abietane diterpenes.[10-12] ^{1}H and ^{13}C NMR spectral data of (**3**) shows an ethyl moiety at δ 3.65 (q, J = 7.0 Hz, H_2-21) and δ 1.12 (t, J = 7.0 Hz, Me-22). The H-5 proton appears as a doublet of doublet, indicating the existence of C-6 methylene protons at δ 2.08 (m, H-6α), and δ 1.99 (dd, J = 10.0, 3.3 Hz, H-6β), which in turn coupled with another methine proton at δ 4.40 (dd, J = 3.3, 1.4 Hz) attributed to H-7, establishing the closure of the ethoxyl group at C-7. The coupling patterns of a double doublet with J value of 1.8 and 8.5 Hz of H-7 permitted the assignment of an axially oriented ethoxyl group of C-7.[2] ^{13}C NMR spectrum of (**3**) showed well-resolved resonances for all 22 carbon atoms. The multiplicity of each carbon atom was determined using ^{13}C-NMR DEPT experiment which revealed the presence of six methyls (including three tertiary at δ (20.13 (C-20), 22.22 (C-18), 33.49 (C-19) and two secondary methyls at δ 16.1 (C-16), 18.82 (C-17)), and one primary methyl at δ 20.46 (C-22), five methylenes at (δ 36.58 (C-1), 19.58 (C-2), 41.98 (C-3), 23.84 (C-6) and 65.60 (C-21)), three methines (including one oxygenated at δ 69.92 (C-7)) and eight quaternary carbons including six aromatic carbons at (δ 113.50 (C-8), 129.41 (C-9), 139.15 (C-11), 155.01 (C-12), 121.15 (C-13) and 157.25 (C-14), and two sp^3 at δ 33.72 (C-4) and 39.72 (C-10). The above data suggested that **(3)** is an abieta-8,11,13-trien type diterpenoid derivative.[20-24] Location of the ethyl moiety at C-7 of (**3**) was deduced from the upfield shift of H-7 (δ 4.40, $^{\delta}\Delta_H$ -0.33 ppm), and the significant downfield shift of C-7 (δ 69.92, $^{\delta}\Delta_C$ + 7.35 ppm) in (**3**) compared to those of H-7 (δ 4.73) and C-7 (δ 62.57) in (**1**), respectively. Consequently, the structure of (**3**) was established as 7α-ethoxy-11, 12, 14-trihydroxy-8, 11, 13-abietatriene. A new example of the isolation of abeitane type diterpene from nature and was named as Salviatane B.

Table 1: ^{1}H and ^{13}CNMR spectral data of compound 3 (400 MHz for ^{1}H, and 100 MHz for ^{13}C, Acetone-d_6) and Compound 5 (500 MHz for ^{1}H and 100 MHz for ^{13}C NMR, DMSO-d_6).

	Compound 3			Compound 5	
Position	**^{1}H (J in Hz)**	**^{13}C**	**DEPT**	**^{1}H (J in Hz)**	**^{13}C***
1	α 1.45, m β 2.71, m	36.58	CH_2	α 1.30, m β 1.50, m	24.00
2	α 1.52, m β 1.73, m	19.58	CH_2	5.87, dd, 10.5, 3.4	66.00
3	α 1.44, m β 1.46, m	41.98	CH_2	6.66, dd, 10.5, 1.9	137.00
4	–	33.72	C	–	139.00
5	α 1.61, d, 12.8	46.39	CH	–	41.00
6	α 2.08, m β 1.99, dd, 13.0, 1.8	23.84	CH_2	α 2.35, m β 1.80, m	40.50
7	4.40, dd, 8.5, 1.8	69.92	CH	5.40, ddd, 4.9, 3.6, 2.1	74.00
8	–	113.50	C	2.25, m	41.50
9	–	129.41	C	1.45, m	32.00
10	–	39.72	C	2.30, ddd, 10.5, 10.5, 6.5	43.00
11	–	139.15	C	α 1.20, m β 1.40, m	30.00
12	–	155.01	C	α 1.90, m β 2.15, m	20.00
13	–	121.15	C	–	131.00
14	–	157.25	C	6.50, d, 1.5	109.00
15	3.18, hept., 7.0	24.88	CH	7.46, t, 1.5	144.00
16	1.23, d, 6.8	16.11	CH_3	7.53, d, 1.5	141.00
17	1.18, d, 6.8	18.82	CH_3	1.15, d, 7.2	12.00
18	0.92, s	22.22	CH_3	–	171.00
19	0.93, s	33.49	CH_3	α 3.94, dd, 7.2, 2.2 β 5.35, d, 7.2	73.00
20	1.22, s	20.13	CH_3	–	170.50
21	3.65, q, 7.0	65.60	CH_2	2.00, s	21.00
22	1.12, t, 7.0	20.46	CH_3	–	–

*Data obtained from HMQC and HMBC.

Compound 4: was obtained as a white crystal solid and gave on TLC violet color with vanillin/H_2SO_4. $(\alpha)^{25}_D$ –25 (c.0.1, $CHCl_3$). It had molecular formula of $C_{41}H_{64}O_3$ as determined by its positive ion ESI-MS at m/z 643 $(M + K)^+$ and from ^{13}C, and DEPT-NMR spectral data (Table 2). Analysis of the IR spectrum of (**4**) suggested that it contained a hydroxyl (3395 cm^{-1}), olefinic (1660, 895 cm^{-1}) and geminal dimethyl (1387, 1367 cm^{-1}) functionalities. The ^{1}HNMR spectrum of (**4**) (Table 2) showed the existence of seven tertiary methyl groups as singlet at δ_H (0.77 (3H, Me-34), 0.82 (3H, Me-31), 0.88 (3H, Me-41), 1.22 (3H, Me-39), 1.23 (3H, Me-40), 1.60 (6H, Me-35, Me-36)), twelve methine proton signals including 6 olefenic functionalities at δ_H 5.35 (brs, H-14), 5.14 (brs, H-19), 5.10 (d, J = 7.2 Hz, H-27), 5.16 (d, J = 7.3 Hz, H-28), 5.91 (dd, J = 17.3, 10.7 Hz, H-32), 5.95 (dd, J = 17.3, 10.7 Hz, H-37). The remaining six methine protons were observed at δ_H 1.10 (m, H-5) 1.99 (m, H-9), 2.05 (m, H-12), 2.04 (dd, J = 4.3, 2.2 Hz, H-17), 1.87 (m, H-21), 1.66 (m, H-25), and fourteen methylene protons including two olefinic signals at δ_H 5.32 and 4.98 (each 1H, dd, J = 17.3, 1.8 Hz, CH_2-33) and δ_H 5.19, 4.95 (each 1H, dd, J = 17.3, 1.8 Hz, CH_2-38). The ^{13}C NMR spectrum (Table 2) confirmed the previous data through the resonances displayed at δ_C 15.99, 16.09, 13.96, 25.84, 17.74, 22.17, 122.28, 125.07, 135.15, 125.75, 147.12, 146.91, 56.14, 51.06, 33.54, 64.04, 51.06, 56.14, and 111.26, respectively. Inspection of the DEPT-^{13}CNMR spectra (135° pulse sequence) also revealed the presence of seven methyls, fourteen methylenes; including 2 vinyl carbons at δ 111.26 (C-33 and C-38), and twelve methines; including 6 sp^2 at δ 122.28 (C-14), 125.07 (C-19), 135.15 (C-27), 125.07 (C-28), 147.12 (C-32), 146.91 (C-37). Among the eight signals of quaternary carbons observed in ^{13}C NMR spectrum, two olefinic at δ_C 136.39 (C-13), 135.43 (C-18) and three oxygenated quaternary at δ_C 73.27 (C-4), 72.82 (C-8 and 22) were recognized. These spectral data and by literature comparisons, compound (**4**) was recognized as a member of *bis*-labdane type diterpene[25-27] and showed the resemblance of the signal multiplicities, chemical shifts and coupling constants observed for the vinyl groups, methines and side chain carbons in both units, suggesting a close structural relationship between them (Figure 1). Comparison of the IR, ^{1}H and ^{13}C NMR spectral data of (**4**) with those of the *bis*-labdane type diterpenoid (Lancelatine and Lanceolatol) isolated from the roots of *Cuuinghamia laceolata*[25] concluded that the difference between (**4**) and Lancelatine and Lanceolatol was the presence of hydroxyl group at C-4 and

Table 2: ^{1}H and ^{13}CNMR spectral data of compound 4 (400 MHz for ^{1}H, and 100 MHz for ^{13}C, Acetone-d_6).

Position	^{1}H (J in Hz)	^{13}C	DEPT	Position	^{1}H (J in Hz)	^{13}C	DEPT
1α	1.66, m	39.90	CH_2	21	1.87, m	50.06	CH
1β	1.76, ddd, 12.7, 4.5, 4.3						
2	1.67, m	19.46	CH_2	22	–	72.82	C
3	1.40, m	43.07	CH_2	23	1.97, m	40.46	CH_2
4	–	73.27	C	24α	2.10, m	27.26	CH_2
				24β	2.08, m		
5	1.01, m	56.14	CH	25	1.66, m	56.14	CH
6α	1.20, m	27.26	CH_2	26	–	33.53	C
6β	1.27, m						
7	2.11, m	40.43	CH_2	27	5.10, d, 7.2	135.15	CH
8	–	72.82	C	28	5.16, d, 7.3	125.75	CH
9	1.99, m	51.06	CH	29α	1.29, m	43.36	CH_2
				29β	1.90, m		
10	–	37.85	C	30	–	37.85	C
11α	1.48, m	23.34	CH_2	31	0.82, s	16.09	CH_3
11β	1.65, m						
12	2.05, m	33.54	CH	32	5.91, dd,17.3, 10.7	147.12	CH
13	–	136.39	C	33α	5.23, dd, 17.3, 1.8	111.26	CH_2
					4.98, dd, 10.7, 1.8		
				33β			
14	5.35, brs	122.28	CH	34	0.77, s	15.99	CH_3
15α	1.16, m	27.46	CH_2	35	1.60, s	17.74	CH_3
15β	2.06, m						
16α	1.95, m	24.45	CH_2	36	1.60, s	22.17	CH_3
16β	1.98, m						
17	2.04, dd, 4.3, 2.2	46.04	CH	37	5.95, dd,17.3, 10.7	146.91	CH
18	–	135.43	C	38α	5.19, dd, 17.3, 1.8	111.26	CH_2
				38β	4.95, dd, 10.7, 1.8		
19	5.14, brs	125.07	CH	39	1.22, s	25.84	CH_3
20	2.00, m	22.11	CH_2	40	1.23, s	22.17	CH_3
				41	0.88, s	13.96	CH_3

methyl group at C-26 in (**4**) versus carboxyl and hydroxymethyl groups in the same position in Lancelatine and Lanceolatol, respectively.

The other difference was the presence of two vinyl and two hydroxyl groups at C-8 and C-22 in (4) versus methyline groups in Lanceolatol.[25] Compound (4) was constructed of two different monomers (12*E*-8-vinyl-12,14-labdane-diene-4,8-diol (unit 1) and 12*E*-8-vinyl-2,12,14-labdane-triene-8-ol (unit 2), and both of them were constructured by Diels-Alder cycloaddition reaction of the two different diterpene monomer units (Figure 2). There are two ways in which the Diels-Alder cycloaddition reaction could occur, either head to head or head to tail. Furthermore, one monomer should act as diene and the other as dienophile in the Diels-Alder cycloaddition reaction. Then there are four possibilities for the structure of compound (4). In the ^{1}H and ^{13}C NMR spectra, the chemical shift difference of two methyline protons (δ_H 5.23 (H-33α) and 4.95 (H-33β)), connected with vinyl carbon at δ_C 111.26 (C-33), were more different than that of two protons attached to δ_C 111.26 (C-38), (δ_H 5.19 (H-38α) and 4.98 (H-38β)). This is because one proton is shielded and the other is deshielded by the double bond between C-18 and C-19. Therefore, the monomer containing the methylene protons at this vinyl carbon (δ_H 5.23 (H-33α), 4.95 (H-33β)), should be the dienophile in the Diels-Alder cycloaddition. The chemical shifts of (C-11 to C-21) in the ^{1}H and ^{13}C NMR spectra (Table 2), showed close similarities to those of Lancelatine, Lanceolatol and Lanceolatic acid,[25,27] confirmed that the link pattern of the (4) was the same as these *bis*-labdane type diterpenoids, and should be head to head Diels-Alder cycloaddition. Based on these spectral data the structure of (4) was established as 12, 15 (22-hydroxy-28-vinyl labdane-27-ene) 8-vinyl-4, 8-labdane-diol, a new natural *bis*-labdane type diterpenoid and was named as Salvianol A. Interestingly, this is the first example of the isolation of a *bis*-labdane type diterpene from *genus Salvia*.

Compound 5: was isolated as colorless prisms, $(\alpha)^{25}_{D}$ - 115° (c.0.20, $CHCl_3$). The molecular formula was deduced as $C_{21}H_{26}O_6$ from the molecular ion peak observed at m/z 374 $(M)^+$ in the EI-MS. The IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}), α, β-unsaturated carbonyl group (1755 cm^{-1}), lactone ring (1760 cm^{-1}), and a furan ring (1490, 876 cm^{-1}) absorptions. The UV spectrum of (**5**) exhibiting a maximum at 240 nm indicated the presence of an α, β-unsaturated carbonyl group. The ^{1}H NMR spectrum of (**5**) (Table 1), exhibited 25 non-exchangeable protons, including one secondary methyl group at δ 1.15 (3H, d, J = 7.2 Hz, H_3-17), one tertiary methyl group at δ 2.00 (3H, s, H_3-21), and four olefinic protons; three were assigned to the β-substituted furan ring[28,29] based on a pattern arising from aromatic resonances at (δ 6.50 (1H, d, J =1.5 Hz, H-14), 7.46 (1H, t, J =1.5 Hz, H-15), and 7.53 (1H, d, J =1.5 Hz, H-16)), and an olefinic proton assigned at δ 6.66 (1H, dd, J =10.5, 1.9 Hz, H-3), as well as two methine proton signals shifted downfield at δ 5.40 (1H, ddd, J = 4.9, 3.6, 2.1 Hz), and 5.87 (1H, dd, J =10.5, 3.4 Hz), were assigned to H-7 and H-2, respectively. The ^{1}H NMR spectrum also showed signals corresponding to an oxymethylene protons at δ 3.94 (1H, dd, J =7.2, 2.2 Hz, H-19α), and 5.35 (1H, d, J = 7.2 Hz, H-19β). The ^{13}C NMR data of (**5**) {obtained from HMQC and HMBC spectra} (Table 1) showed the presence of 21 carbon atoms in (**5**) from which 16 are protonated (two methyls at δ 12.00 (C-17), and 21.00 (C-21), five methylenes including an oxygenated carbon at δ 73.00 (C-19), five methines including two oxygenated carbons at δ 66.00 (C-2) and 74.00 (C-7), and four olefinic carbons at δ 109.0 (C-14), 131.0 (C-13), 141.0 (C-16), 144.0 (C-15)), arising from the β-substituted furan ring.[29-31] The remaining five carbons are quaternary including two carbonyls at δ 170.5 (C-20), and 171.0 (C-18). The methyl group at (δ 21.0) and the ester carbonyl group at (δ 170.5) suggested the presence of an acetoxyl moiety in the molecule of (**5**). The olefinic carbons and the two carbonyl groups accounted for five of the nine unsaturations, thus implying that (**5**) consisted of four-ring system with a structure related to a tetracyclic clerodane diterpenoid[29,32] and was nearly similar to that of Salvigresin isolated previously from *Salvia greggii*.[33] On the bases of the interpretation of HMQC which gave the

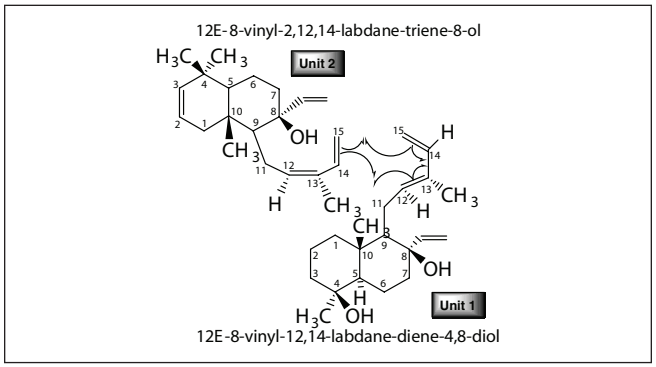


Figure 2: The structure of the two units of 4.

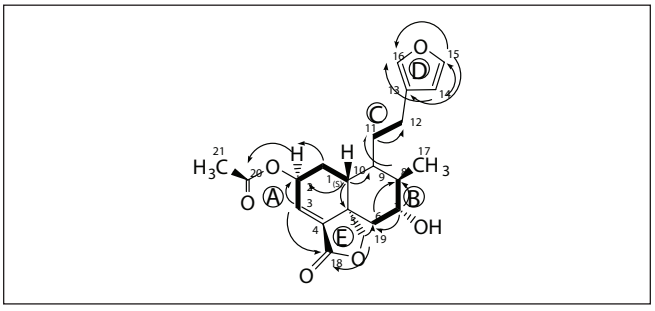


Figure 3: HMQC and HMBC correlations of partial structure of Salviaclerodan A.

corresponding carbon assignments and HMBC experiments, which used for detection of quaternary carbon atoms and all possible two-and three-bond inter-and intra-residue correlations. HMQC of (**5**) suggested the presence of five partial structures; A-E (Figure 3), in addition to the quaternary carbon at δ 41.0 (C-5), two methyl groups at δ 12.0 (CH_3-17) and 21.0 (CH_3-21) and the above two carbonyl carbons. The connectivity of these partial structures was deduced from HMBC spectrum of (**5**). The methyl group singlet at δ 2.0 (H_3-21) showed correlation to the carbonyl carbon at δ 170.5 (C-20), the methine proton at δ 2.30 (H-10) was correlated to the carbon at δ 66.0 (C-2) of segment **A**. The methine carbon at δ 41.50 (C-8) of the segment **B** and the methylene carbon at δ 30.0 (C-11) of segment **C**. The olefinic proton at δ 6.66 (H-3) of the segment **A** with carbonyl group at δ 171.0 (C-18). The methylene protons at δ 3.94 and 5.35 (H_2-19) of the segment **C** were correlated to the quaternary carbons at δ 139.00 (C-4), 41.0 (C-5) and 171.00 (C-18) of the segment **E**, respectively. On the other hand the connectivities observed between H-14 (δ 6.50) and the olefinic carbons at δ 141.0 (C-16) and 144.0 (C-15); H-15 (δ 7.46) and the olefinic carbon at δ 131.0 (C-13), and between H-16 (δ 7.53) and (C-13 and C-16) of the segment **D**, confirmed the presence of *β*-substituted furan ring.[29-32, 34, 35] The correlations observed between H-2 (δ 5.87) and carbonyl group at δ 170.50 (C-20) indicated that the acetyl group was attached at C-2. The relative stereochemistry of the chiral centers in structure (**5**) could be assigned readily on the basis of signal multiplicities, chemical shifts and coupling constants in the ^{1}H NMR spectrum. Large coupling constants between H-2 and H-10 (10.5 Hz) required that H-2 and H-10 be found in axial α-and β-orientations, respectively. Furthermore, diaxial couplings between H-7 and H-8 (4.9 Hz) required that both hydroxyl and methyl groups must be equatorial. Therefore H-7 and H-8 must be in α-and β-orientations, respectively. The most difficult stereochemical assignment was at H-9. Observation of the multiplet couplings between H-9 and the two H-11 hydrogens, implied that H-9 could be in α-or β-orientation. The structure of (**5**) was closely related to salvigresin isolated from *Salvia gregii*.[25, 28] The most notable difference with the salvigresin is the missing of an acetyl, a methyl, and a hydroxyl group from positions, 7, 9 and 12, respectively in (**5**). Therefore by use of a combination of ^{1}H NMR, HMQC and HMBC, the structure of **(5)** was established as (5*S*, 7*R*, 8*R*, 9*S*, 10*R*)-2*S*-acetoxy-15, 16-epoxy-cleroda-3, 13(16), 14-trien-18, 19-olide, a new natural clerodane type diterpene and was named as Salviaclerodan A.

Compound 11: was isolated as a light yellow powder and gave on TLC violet and yellow color with vanillin/H_2SO_4 and pauly's reagents,[36] respectively. $(\alpha)^{25}_D$ - 10.7^0 (c 1.0, $CHCl_3$). The molecular formula of (**11**) was determined to be $C_{18}H_{14}O_7$ on the bases of a positive FAB-MS m/z 343 $(M + H)^+$, along with a significant fragment peak at *m/z* 307 $(M + H - 2H_2O)^+$. The UV absorptions at 291 and 325 nm showed that (**11**) has a conjugated aromatic system. Absorption bands at 3430 cm^{-1} (hydroxyl), 1710 cm^{-1} (conjugated carbonyl) and 1590 cm^{-1} (aromatic), functional groups were suggested by IR spectrometry. 1D (^{1}H NMR) spectrum, in conjunction with detailed two dimensional (2D) analysis by the ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear multiquantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) (Table 3) were performed on compound (**11**) and revealed the presence of two aromatic rings, two pairs of *trans* olefinic protons and two carboxylic groups (a carbon signal at δ_C 168.50, reasonably assigned to a carboxylic carbonyl carbon).

The ^{1}H NMR data also revealed the presence of ten protons (five proton signals integrated for two protons each) in addition to a singlet peak integrated for two protons at δ 10.99, confirmed (**11**) is a dimmeric structure of cinnamic acid derivative and indicating that the linkage between the two acid moieties by one ether bond. Examination of assignments (signal multiplicities, chemical shifts and coupling constant) in the ^{1}H NMR spectrum achieved for all protons of (**11**) were in agreement for those reported for two (*E*)-caffeic acid moieties.[37-39] Two *trans*-olefinic protons (AB-system, J =16 Hz) at δ 7.38 (2H, H-7, H-7') and δ 6.15 (2H, H-8, H-8'), six aromatic protons belonging to two set of typical ABX- spin system; (signals for 1,3,4-trisubstituted aromatic ring protons were observed at δ 7.11 (2H, d, J = 1.9 Hz, H-2, H-2'), δ 6.75 (2H, d,

Table 3: ^{1}H and ^{13}C NMR spectral data of compound 11 (500 MHz for ^{1}H, and 100 MHz for ^{13}C, DMSO-d_6).

Position	^{1}H (*J* in Hz)	COSY	^{13}C NMR*	HMBC
1,1'	–	–	126.50	–
2,2'	7.11, d, 1.9	–	115.00	C-3, 3' ; C-4, 4'; C-6, 6'
3,3'	–	–	148.50	–
4,4'	–	–	146.50	–
5,5'	6.71, d, 8.2	H-6, 6'	116.50	C-1, 1'; C-3, 3'; C-4, 4'
6,6'	6.92, dd, 8.2, 1.9	H-5, 5'	121.00	C-2, 2'; C-4, 4'
7,7'	7.38, d, 16.0	H-8, 8'	142.50	C-2, 2'; C-6, 6'; C-9, 9'
8,8'	6.15, d, 16.0	H-7, 7'	116.00	C-1, 1'; C-9, 9'
9,9'	10.99, brs	–	168.50	–

*Data obtained from HMQC and HMBC.

J = 8.2 Hz, H-5, H-5') and δ 6.92 (2H, dd, *J* =8.2, 1.9 Hz, H-6, H-6')), meaning that one of C-3 or C-4 hydroxyl groups were substituted in the dimeric structure of (*E*)-caffeic acid . These observations were also confirmed by ^{1}H-^{1}H-COSY-NMR spectrum which showed connectivities between H-7 and H-8 and between H-7' and H-8'. Furthermore, protonated carbons and quaternary carbons were assigned by HMBC. Thus long range correlations between the (H-7 (δ 7.38) and C-7 (142.50)/C-8 (116.00)) unsubstituted double bond and the carbons of the unsubstituted caffeic acid moiety δ 115.00, (C-2) and δ116.50 (C-6), showed that the caffeic acid units was involved only by an ether linkage. Moreover, the H-7/H-8 and H-7'/H8' are also coupled with acid carbonyl carbons C-9/ C-9' which resonated at δ 168.50. Short and long range correlations allowed to distinguish the C-1, C-2, C-3, C-4, C-5 and C-6 signals of each residual phenolic rings, and to attribute H-2 at (δ 7.11, d, *J* =1.9 Hz), H-5 at 6.71 (d, *J* = 8.2 Hz) and H-6 at δ (6.92, dd, *J* = 8.2, 1.9 Hz) in each ring. Long range correlations of both H-5 protons with both substituted C-3 carbons indicated that the linkage between caffeic acid moieties was 3, 3' bond. FAB-MS of (**11**) confirmed also the location of the dimeric structure on C-3, C-3' by a fragment at m/z 307 $(M + H - 2H_2O)^+$, clearly confirmed the loss of two molecules of water from two free carboxylic acids. Additionally C-3/C-3' signal of (**11**) was shifted downfield to (δ 148.50) in comparison with those reported for unsubstituted caffiec acid C-3 δ 144.50 (Δ + 4.0 ppm) dimmer and tetramer (Yannanic acid) derived by oxidative coupling of two molecules of rosmarininc acid isolated previously from *Salvia yannanesis*.[39] Therefore the structure of (**11**) was a new caffeic acid dimmer, and was named 3, 3'-didehydrodicaffeic acid.

Results of cytotoxic activity: Cell lines SNU-398, Hep G2, PLC/PRF/5, HT-1376, UM-UC-3, Hs 388.T, Hs 751.T were the most sensitive of all lines examined to the activities of *S. lanigra* and *S. splendens* extracts (MeOH, Acetone and *n*-BuOH) (Table 4). These extracts showed also moderate cytotoxic activities when tested against ovary adenocarcinoma (NIH:OVCAR-3 and SK-OV-3). Cell line MES-SA/MX2 was the least sensitive of all lines examined to the activities of these extracts. However, neither extract, showed any cytotoxic activity (>100) against kidney hypernephroma (SW 156), kidney carcinoma (A-498) and uterine sarcoma (MES-SA) cells in a concentration-dependent manner at the recommended NCI (USA) doses.[40] The diterpenes containing 11, 14-dione; Horminone (**1)** and 7-O-ethoxy-horminone (**2**), 11, 12-dihydroxyl groups; salviatane B (**3**), *bis*-labdane type diterpene; salvianol A (**4**) and 7-hydroxy clerodane derivative; salviaclerodan A (**5**) showed the most potent cytotoxic activities of the active compounds isolated and identified, showing significant activity (ED_{50} = 2.25 to 18.70 μg/mL), with human liver hepat-cellular carcinoma (SNU-398, Hep G2), human liver hepatoma (PLC/PRF/5), urinary bladder transitional cell carcinoma (UM-UC-3) and Lymph node Hodgkin's disease; Hodgkin's lymphoma (Hs 388.T, Hs 751.T) cell lines (Table 5). Salvigenin (**9**) was generally more potent than apigenin (**10**) and less potent than diterpenes **(1-5)**, possessing the most cytotoxic activities with human liver hepato-cellular carcinoma (SNU-398, Hep G2), human liver hepatoma (PLC/PRF/5), urinary bladder carcinoma (HT-1376), stomach gastric carcinoma (Hs 746T, Hs 740.T), ovary adenocarcinoma (NIH:OVCAR-3, SK-OV-3) (Table 5).

Results of antioxidant activity: Among the three different extracts, acetone extract expected to have the highest activity in DPPH• quenching (65.5%) while MeOH extract (59.8%) and *n*-BuOH extract (57.2%) showed slightly less DPPH• activity than D, L-α-tocopherol (72.3%) but slightly more than BHT (52.6%) (**Table 6**). The results obtained with compounds (**1-5**, **9** and **10**); salviatane B (**3)** (82.4 %), Horminone (**1)** (78.8%), and 7-O-ethoxy-horminone (**2)** (74.5%) showed the highest DPPH• quenching than those of such typical antioxidants D, L-α-tocopherol (72.3%) and BHT (52.6%). Salvigenin (**9)** (58.5%) and apigenin (**10)** (55.5) showed slightly less DPPH• activity than D, L-α-tocopherol (72.3%), but highest than BHT (52.6%). Salvianol A (**4)** and salviaclerodan A (**5**) showed less DPPH• quenching activity than D, L-α-tocopherol and BHT (Table 6).

Horminone (1): A pale yellow amorphous solid; UV λmax (MeOH) nm: 270, 402; IR υmax (KBr) cm^{-1}: 3395, 1665, 1640, 1607; ^{1}H NMR (400 MHz, acetone-d_6) δ 4.73 (1H, *dd*, *J* = 3.6, 1.4 Hz, H-7), 3.65 (2H, m, H2-21), 3.17 (1H, hept, *J* = 7.1 Hz, H-15), 1.23 (3H, s, H3-20), 1.21 (3H, *d*, *J* = 7.1 Hz, H_3-16), 1.17 (3H, d, J= 7.1 Hz, H_3-17), 0.94 (3H, s, H_3-19), 0.91 (3H, s, H_3-18); ^{13}C NMR (100 MHz, acetone-d_6) δ 188.67 (C-14, s), 185.03 (C-11, s), 153.16 (C-12, s), 147.85 (C-9, s), 143.76 (C-8, s), 124.51 (C-13, s), 62.57 (C-7, d), 46.17 (C-5, d), 41.99 (C-3, t), 39.85 (C-10, s), 36.58 (C-1, t), 33.63 (C-4, s), 33.55 (C-19, q), 27.20 (C-6, t), 24.69 (C-15, d), 22.13 (C-18, q), 20.28 (C-20, q), 20.16 (C-17, q), 19.56 (C-2, t), 18.74 (C-16, q); positive ESIMS *m/z* 333 $[M + H]^+$, 1030 $[3M + H + K]^+$.

7-*O*-ethylhorminone (2): A yellow amorphous powder; UV λmax (MeOH) nm: 273, 402; IR υ_{max} (KBr) cm^{-1}: 3400, 1665, 1645; ^{1}H NMR (400 MHz, acetone-d_6) δ 4.40 (1H, *dd*, *J* = 3.4, 1.8 Hz, H-7), 3.18 (1H, hept, *J* = 7.0 Hz, H-15), 1.23 (3H, s, H_3-20), 1.21 (3H, *d*, *J* = 7.0 Hz, H_3-16), 1.17 (3H, *d*, *J* = 7.0 Hz, H_3-17), 1.11 (3H, *t*, *J* = 7.0 Hz, H_3-22), 0.93 (3H, s, H_3-19), 0.92 (3H, s, H_3-18); ^{13}C NMR (100 MHz, acetone-d_6) δ 186.98 (C-14, s), 185.00 (C-11, s), 152.79 (C-12, s), 148.04 (C-9, s), 142.18 (C-8, s), 124.78 (C-13, s), 69.88 (C-7, d), 65.63 (C-21, t), 46.35 (C-5, d), 41.93 (C-3, t),

Table 4: Cytotoxicity of *S. lanigra* and *S. splendens* extracts against selected Liver, Kidney, Urinary bladder, Stomach, Ovary, Lymph node, Spleen and Uterus tumor cell lines.

	Salvia lanigra extracts ED_{50} (µg/mL)			*Salvia splendens* extracts ED_{50} (µg/mL)		
Cell Line	**MeOH**	**Acetone**	***n*-BuOH**	**MeOH**	**Acetone**	***n*-BuOH**
Liver hepatocellular carcinoma						
SNU-398	14.22 (± 0.12)	9.83 (± 0.05)	10.04 (± 0.08)	16.56 (± 0.18)	7.42 (± 0.05)	9.14 (± 0.05)
Hep G2	8.56 (± 0.05)	6.95 (± 0.03)	8.12 (± 0.05)	7.82 (± 0.09)	9.15 (± 0.07)	12.16 (± 0.10)
Liver hepatoma						
PLC/PRF/5	13.35 (± 0.10)	10.66 (± 0.08)	18.10 (± 0.15)	17.50 (± 0.16)	19.85 (± 0.18)	17.58 (± 0.15)
Kidney hypernephroma						
SW 156	>100	>100	>100	>100	>100	>100
Kidney carcinoma						
A-498	>100	>100	>100	>100	>100	>100
Urinary bladder carcinoma						
HT-1376	34.92 (± 0.28)	19.78 (± 0.17)	22.73 (± 0.20)	43.12 (± 0.30)	17.85 (± 0.15)	20.73 (± 0.20)
Urinary bladder transitional cell carcinoma						
UM-UC-3	20.18 (± 0.18)	23.45 (± 0.22)	26.12 (± 0.25)	16.35 (± 0.15)	15.25 (± 0.15)	20.14 (± 0.20)
Stomach gastric carcinoma						
Hs 746T	>100	65.91 (± 0.58)	>100	85.60 (± 0.75)	78.48 (± 0.70)	90.85 (± 0.80)
Hs 740.T	>100	>100	>100	>100	80.15 (± 0.76)	87.45 (± 0.80)
Ovary adenocarcinoma						
NIH:OVCAR-3	66.15 (± 0.55)	58.40 (± 0.45)	77.92 (± 0.68)	72.30 (± 0.60)	85.10 (± 0.75)	88.12 (± 0.77)
SK-OV-3	70.95 (± 0.60)	65.78 (± 0.50)	>100	77.18 (± 0.65)	60.90 (± 0.45)	93.24 (± 0.80)
Lymph node (Hodgkin's lymphoma)						
Hs 388.T	14.95. (± 0.15)	8.50 (± 0.05)	16.13 (± 0.16)	18.25 (± 0.16)	11.45 (± 0.08)	10.33 (± 0.08)
Hs 751.T	7.93 (± 0.05)	5.65 (± 0.03)	7.72 (± 0.04)	9.12 (± 0.08)	5.18 (±0.03)	6.55 (± 0.05)
Uterus (uterine sarcoma)						
MES-SA	>100	>100	>100	>100	>100	>100
MES-SA/ MX2	75.38 (± 0.68)	78.78 (± 0.70)	83.90 (± 0.85)	81.17 (± 0.60)	85.53 (± 0.80)	88.85 (± 0.85)

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all value

Table 5: Cytotoxicity of isolated compounds (1-5, 9 and 10) from *S. lanigra* and *S. splendens* against selected Liver, Kidney, Urinary bladder, Stomach, Ovary, Lymph node, Spleen and Uterus tumor cell lines.

Cell Line	ED_{50} (µg/mL)						
	1	2	3	4	5	9	10
Liver hepatocellular carcinoma							
SNU-398	8.28 (± 0.05)	9.03 (± 0.05)	3.39 (± 0.02)	5.80 (± 0.05)	11.75 (± 0.08)	66.50 (± 0.50)	10.62 (± 0.06)
Hep G2	5.68 (± 0.03)	5.70 (± 0.04)	2.25 (± 0.02)	12.54 (± 0.15)	15.95 (± 0.10)	73.11 (± 0.55)	17.24 (± 0.12)
Liver hepatoma							
PLC/PRF/5	4.95 (± 0.02)	6.82 (± 0.05)	3.10 (± 0.01)	11.97 (± 0.10)	18.55 (± 0.08)	82.25 (± 0.66)	21.66 (± 0.14)
Kidney hypernephroma							
SW 156	>100	>100	>100	>100	>100	76.50 (± 0.50)	50.52 (± 0.32)
Kidney carcinoma							
A-498	>100	>100	>100	>100	>100	55.98 (± 0.50)	43.87 (± 0.26)
Urinary bladder carcinoma							
HT-1376	22.19 (± 0.15)	20.08 (± 0.10)	17.35 (± 0.10)	27.03 (± 0.15)	32.65 (± 0.18)	49.88 (± 0.30)	29.38 (± 0.18)
Urinary bladder transitional cell carcinoma							
UM-UC-3	16.55 (± 0.12)	13.30 (± 0.08)	7.40 (± 0.04)	10.14 (± 0.08)	25.35 (± 0.13)	>100	>100
Stomach gastric carcinoma							
Hs 746T	>100	80.55 (± 0.68)	>100	>100	75.90 (± 0.65)	>100	34.60 (± 0.22)
Hs 740.T	> 100	>100	>100	64.95 (± 0.58)	70.87 (± 0.60)	91.59 (± 0.76)	28.11 (± 0.16)
Ovary adenocarcinoma							
NIH:OVCAR-3	75.14 (± 0.65)	62.55 (± 0.50)	58.17 (± 0.45)	80.85 (± 0.80)	78.65 (± 0.70)	69.95 (± 0.55)	6.78 (± 0.03)
SK-OV-3	70.95 (± 0.60)	>100	80.25 (± 0.75)	73.50 (± 0.75)	>100	>100	20.74 (± 0.11)
Lymph node (Hodgkin's lymphoma)							
Hs 388.T	18.70. (± 0.15)	12.90 (± 0.06)	5.15 (± 0.03)	8.20 (± 0.05)	21.25 (± 0.18)	96.56 (± 0.70)	48.99 (± 0.15)
Hs 751.T	4.90 (± 0.05)	8.54 (± 0.03)	3.10 (± 0.04)	6.74 (± 0.05)	25.60 (±0.03)	83.15 (± 0.65)	70.13 (± 0.53)
Uterus (uterine sarcoma)							
MES-SA	>100	>100	>100	>100	>100	>100	66.70 (± 0.15)
MES-SA/ MX2	>100	>100	>100	>100	>100	>100	53.90 (± 0.53)

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all value

Table 6: Effects of *Salvia lanigra* extracts (MeOH, Acetone and *n*-BuOH), isolated compounds (1-5, 9 and 10) and positive controls on the *in vitro* Free Radical Generation.

Bioassay	DPPH• % decoloration Sample 1000 µg/ml	Bioassay	DPPH• % decoloration Sample 1000 µg/ml
MeOH extract	59.8 ± 2.95	Compound 4	35.8 ± 1.50
Acetone soluble	65.5 ± 3.10	Compound 5	42.3 ± 1.85
n-BuOH soluble	57.2 ± 2.75	Compound 9	55.5 ± 2.44
Compound 1	78.8 ± 3.35	Compound 10	58.5 ± 2.82
Compound 2	74.5 ± 3.15	D and L-α-tocopherol	86.4 ± 3.50
Compound 3	82.4 ± 3.30	BHT	62.4 ± 2.75

Values are presented as mean + SE of 3-test sample observation.
$P < 0.05$ for all values.

39.76 (C-10, s), 36.55 (C-1, t), 33.71 (C-4, s), 33.47 (C-19, q), 24.87 (C-15, d), 23.77 (C-6, t), 22.20 (C-18, q), 20.37 (C-22, q), 20.05 (C-20, q), 19.55 (C-2, t), 18.81 (C-17, q), 16.08 (C-16, q); positive ESIMS *m/z* 360 $[M]^+$, 361 $[M + H]^+$, 315 $[M - CH_3CH_2O]^+$, 297 $[M - CH_3CH_2O - H_2O]^+$, 255 $[M + H - isopropyl - H_2O]^+$.

Salviatane B (3): A yellow amorphous solid; UV λmax (MeOH) nm: 280, 245; IR υ_{max} (KBr) cm^{-1}: 3420, 1615, 1580, 1502; ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data are listed in Table 1; ESIMS *m/z* 363 $[M + H]^+$, 317 $[M + H - CH_3CH_2O]^+$.

Salvianol A (4): A white crystal solid; $[\alpha]^{25}_D$ - 25 (c.0.1, $CHCl_3$); UV λ_{max} (MeOH) nm: 280, 245; IR υ_{max} (KBr) cm^{-1}: 3395, 1660, 1387, 1367, 895; ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data are listed in Table 2; ESIMS *m/z* 643 $[M + K]^+$.

Salviaclerodan A (5): A colorless prisms; $[\alpha]^{25}_D$ - 115° (c.0.20, $CHCl_3$); UV λmax (MeOH) nm: 240; IR υ_{max} (KBr) cm^{-1}: 3450, 1760, 1755, 1490, 876; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data are listed in Table 3; EIMS *m/z* 374 $[M]^+$.

Ursolic acid (6): An amorphous solid [MeOH]; IR υ_{max} (KBr) 3455, 2955, 1710, 1632, 1160 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.99 (1H, brs, H-12), 2.92 (1H, *dd*, *J* = 10.3, 4.5 Hz, H-3), 1.08 (3H, s, H_3-27), 0.89 (3H, s, H_3-23), 0.84 (3H, s, H_3-25), 0.74 (3H, s, H_3-26), 0.70 (3H, s, H_3-24), 0.68 (3H, *d*, *J* = 6.8 Hz, H_3-29), 0.63 (3H, *d*, *J* = 6.8 Hz, H_3-30); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 179.22 (C-28, *s*), 137.76 (C-13, *s*), 124.17 (C-12, *d*), 76.57 (C-3, *d*), 54.59 (C-5, *d*), 52.19 (C-18, *d*), 46.98 (C-9, *d*), 45.53 (C-17, *s*), 41.50 (C-14, *s*), 39.23 (C-8, *s*), 38.95 (C-19, *d*), 38.66 (C-20, *d*), 38.32 (C-1, *t*), 38.20 (C-4, *s*), 36.46 (C-10, *s*), 36.40 (C-22, *t*), 32.49 (C-7, *t*), 30.17 (C-21, *t*), 29.76 (C-23, *q*), 28.30 (C-15, *t*), 27.49 (C-2, *t*), 26.67 (C-16, *t*), 23.68 (C-27, *q*), 23.00 (C-30, *q*), 22.70 (C-11, *t*), 17.80 (C-6, *t*), 16.61 (C-26, *q*), 16.52 (C-29, *q*), 15.29 (C-24, *q*), 14.92 (C-25, *q*); EIMS *m/z* 456 $[M]^+$, 248, 203.

Oleanolic acid (7): An amorphous solid [MeOH]; IR υ_{max} (KBr) 3430, 2950, 1695, 1640 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 5.15 (1H, brs, H-12), 2.99 (1H, dd, *J* = 10.0, 5.0 Hz, H-3), 1.08 (3H, s, H_3-27), 0.88 (3H, s, H_3-30), 0.86 (6H, s, H_3-23, 25), 0.83 (3H, s, H_3-29), 0.71 (3H, s, H_3-26), 0.65 (3H, s, H_3-24); ^{13}C NMR (100.0 MHz, DMSO-d_6) δ 178.58 (C-28, *s*), 143.83 (C-13, *s*), 121.49 (C-12, *d*), 76.79 (C-3, *d*), 54.77 (C-5, *d*), 47.06 (C-9, *d*), 45.67 (C-17, *s*), 45.44 (C-19, *t*), 42.12 (C-14, *s*), 41.80 (C-18, *d*), 41.30 (C-8, *s*), 38.37 (C-4, *s*), 38.03 (C-1, *t*), 36.59 (C-10, *s*), 33.30 (C-21, *t*), 32.82 (C-29, *q*), 32.39 (C-22, *t*), 32.07 (C-7, *t*), 30.39 (C-20, *s*), 28.22 (C-23, *q*), 27.19 (C-2, *t*), 26.94 (C-15, *t*), 25.58 (C-27, *q*), 23.36 (C-30, *q*), 22.59 (C-16, *t*), 22.49 (C-11, *t*), 18.00 (C-6, *t*), 16.84 (C-26, *q*), 16.02 (C-24, *q*), 15.09 (C-25, *q*); EIMS *m/z* 456 $[M]^+$, 248, 203.

β-sitosterol (8): A colorless needles; IR υ_{max} (KBr) cm^{-1}: 3450, 1635, 1160; ^1H NMR (300 MHz, DMSO-d_6) δ 5.20 (1H, *m*, H-6), 3.25 (1H, *m*, H-3), 1.16 (3H, *s*, H_3-19), 0.88 (3H, *t*, *J* = 7.0 Hz, H_3-29), 0.84 (3H, *d*, *J* = 6.0 Hz, H_3-21), 0.74 (3H, *d*, *J* = 6.6 Hz, H_3-27), 0.72 (3H, *d*, *J* = 6.6 Hz, H_3-26), 0.58 (3H, *s*, H_3-18); EIMS *m/z* 414 $[M]^+$.

Salvigenin (9): A yellowish-white amorphous powder; UV λ_{max} (MeOH) nm: 275, 340; IR υ_{max} (KBr) cm^{-1}: 3410, 1700, 1585; ^1H NMR (400 MHz, acetone-d_6) δ 12.90 (1H, *brs*, 5-OH), 8.03 (2H, *d*, *J* = 8.6 Hz, H-2`,6`), 7.14 (2H, *d*, *J* = 8.6 Hz, H-3`,5`), 6.85 (1H, *s*, H-3), 6.71 (1H, *brs*, H-8), 3.98 (3H, *s*, 6-OMe), 3.91 (3H, *s*, 7-OMe), 3.80 (3H, *s*, 4'-OMe); ^{13}C NMR (100 MHz, acetone-d_6) δ 183.48 (C-4), 164.95 (C-2), 163.77 (C-5), 160.10 (C-4'), 154.06 (C-9), 153.95 (C-7), 133.52 (C-6), 129.06 (C-2', 6'), 124.30 (C-1'), 115.39 (C-3', 5'), 106.53 (C-10), 104.43 (C-3), 91.92 (C-8), 60.50 (6-OMe), 56.79 (7-OMe), 55.99 (4'-OMe); positive ESIMS *m/z* 329 $[M + H]^+$, 679 $[2M + Na]^+$, 1007 $[3M + Na]^+$.

Apigenin (10): A yellowish-white amorphous powder; UV λ_{max} (MeOH) nm: 271, 337; IR υ_{max} (KBr) cm^{-1}: 3430, 1710, 1595; ^1H NMR (400 MHz, DMSO-d_6) δ 12.98 (1H, *brs*, 5-OH), 10.50 (1H, *brs*, 4'-OH), 7.90 (2H, *d*, *J* = 8.8 Hz, H-2',6'), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3',5'), 6.72 (1H, *s*, H-3),

6.40 (1H, *brs*, H-8), 6.10 (1H, *brs*, H-6); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.48 (C-4), 164.95 (C-2), 161.89 (C-5), 157.80 (C-4'), 158.08 (C-9), 164.75 (C-7), 98.50 (C-6), 128.00 (C-2', 6'), 128.30 (C-1'), 116.00 (C-3', 5'), 103.95 (C-10), 102.50 (C-3), 94.00 (C-8); negative-ion MALDI-TOFMS *m/z* 270 $[M]^+$, 269 $[M - H]^+$, 538 $[2M - 2H]^+$.

3,3'-dehydrodicaffeic acid (11): A light yellow powder; $[\alpha]^{25}_D$ - 10.7^0 (c 1.0, $CHCl_3$); UV λ_{max} (MeOH) nm: 325, 291; IR υ_{max} (KBr) cm^{-1}: 3430, 1710, 1590; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data are listed in Table 4; FABMS *m/z* 343 $[M + H]^+$, 435 [M + glycerol]$^+$, 307 $[M + H - 2H_2O]^+$.

ACKNOWLEDGEMENT

Thanks are due to Prof. John, P. N. Rosazza, Head, Division of Medicinal and Natural Products Chemistry and Director of the Center for Biocatalysis and Bioprocessing, College of Pharmacy, University of Iowa, Iowa City, lA 52242 USA, for financial support of spectroscopic data and cytotoxic and antioxidant activity.

REFERENCES

1. Hedge IC. A global survey of the biogeography of the Labiatae. In: Advances in Labiatae Science, (R.M. Harley, T. Reynolds, eds.), Royal Botanic Gardens Kew; 1992. P. 7-17.
2. Chang HM, Cheng KP, Choang TF, Chow HF, Chui KY, Hon PM, et al. Structure nelucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* Bunge (Danshen). Journal of Organic Chemistry. 1990; 55:3537-3543.
3. Zhang KQ, Bao Y, Wu P, Rosen RT, Ho CT. Antioxidative components of tanshen (Salvia miltiorrhiza Bunge). Journal of Agricultural and Food Chemistry. 1990; 38:1194-1197.
4. Lu Y, Foo LY. Polyphenolics of Salvia—a review. Phytochemistry. 2002; 59:117-140.
5. Li LN. Biologically active components from traditional Chinese medicines. Pure and Applied Chemistry. 1998; 70:547-554.
6. Hosny M, Rosazza JPN. New Isoflavone and Triterpene Glycosides from Soybeans. J. Nat. Prod. 2002; 6:805-813.
7. Goren N, Woerdendag HJ, Bozok-Johansson C. Cytotoxic and Antibacterial Activities of Sesquiterpene Lactones Isolated from *Tanacetum praeteritum* subsp. *praeteritum*. Planta Med. 1996; 62: 419-422.
8. Woodson RR. Statistical Methods for the Analysis of Biochemical Data. Series in Probability and Mathematical Statistics; Wiley: New York; 1987. p. 315-316.
9. Hosny M, Rosazza JPN. Novel Oxidations of (+)-Catechin by Horseradish Peroxidase and Laccase J. Agric. Food Chem. 2002; 50:*5639-5645*.
10. Kolak U, Birman H, Ulubelen A, Ari S, Hasancebi S. Cardioactive Diterpenoids from the Roots of *Salvia amplexicaulis*. Planta Med. 2001; 67:761-763.
11. Ulubelen A, Toprti G, Tereni B. Abietna diterpenoids from the roots of *Salvia cryptantha*. Phytochemistry. 1987; 26 (5):1534-1535.
12. Ulubelen A, Birman H, Oksiiz S, Topcu G, Kolak U, Asli Barla a, *et al*. Cardioactive Diterpenes from the Roots of *Salvia eriophora*. Planta Med. 2002; 68:818-821.
13. Topcu G, Ulubelen A. Structure elucidation of organic compounds from natural sources using 1D and 2D NMR technique. Journal of Molecular Structure. 2007; 834–836:57-73.
14. Takahashi H, Hirata S, Minami H, FukuyamaY. Triterpene and flavanone glycoside from *Rhododendron simsii*. Phytochemistry. 2001; 56 (8):875-879.
15. Saba Mughal MH, Ali M, Iqbal M, Srivastava PS. A steryl ester from *Lepidium sativum*. Phytochemistry. 1999; 50:1375-7.
16. Tsuneatsu N, Hikaru O. Studies on the Constituents of Aster scaber THUNB. III. Structures of Scaberosides B_7, B_8 and B_9, Minor Oleanolic Acid Glycosides Isolated from the Root. Chem. Pharm. Bull. 1992; 40 (4):886-888.
17. Agrawal PK. Carbon-13 NMR of Flavonoids, Elsevier Oxford-New-York; 1989. P. 96-116.
18. Topcu G, Ulubelen A, Tam TCM, Tao-Che C. Sesterterpenes and other constituents of *Salvia yosgadensis*. Phytochemistry. 1996; 42 (4):1089-1092.
19. Ulubelen A, Uygur I. flavonoidal and other compounds of *salvia aethiopis*. Planta Med. 1976; 29:318-320.
20. Dentali SJ, Hoffmann JJ. 16-hydroxycarnosic acid, a diterpene from *Salvia apiana*. Phytochemistry. 1990; 29 (3):993-994.
21. Luis JC, Conzalez AG, Andres LS, Mederos S. Diterpenes from *in vitro*-grown *Salvia canariensis*. phytochemistry. 1992; 31(9):3272-3273.
22. Luis GJ, Grillo TA. Abietane diterpenes from *Salvia munzii*. Phytochemistry. 1993; 34 (3):863-864.
23. Lin TJ, Liu GT, Liu Y, Xu GZ. Protection by salvianolic acid A against adriamycin toxicity on rat heart mitochondria. Free Radical Biol. Med. 1992; 12:347-351.
24. Amaro-Luis J, Herrera R, Luis JG. Abietane diterpenoids from *Salvia chinopeplica*. Phytochemistry. 1998; 47:895-897.
25. Du J, Chen R, Yu DQ. A New Bislabdane-Type Diterpenoid from the Roots of *Cunninghamia lanceolata*. J. Nat. Prod. 1999; 62:1200-1201.
26. Du J, Wang M, Chen R, Yu DQ. Two New Bislabdane-Type Diterpenoids and Three New Diterpenoids from the Roots of *Cunninghamia lanceolata*. Planta Med. 2001; 67:542-547.
27. David JP, David JM, Yang S, Cordell A. A bis-labdenic diterpene from *Moldenhawera nutans*. Phytochemistry. 1998; 50:443-447.
28. Ortega A, Cardenas J, Cage DA, Maldonado E. Abietane and clerodane diterpenes from *Salvia regla*. Phytochemistry. 1995; 39 (4), 931-933.
29. Bisio AD, Tommasi N, Romussi G. Diterpenoids from *Salvia wagneriana*, Planta Med. 2004;70:452-457.
30. Petersen M, Simmonds MS. Rosmarinic acid. *Phytochemistry*. 2003; 62:121-125.
31. Yang H, Sun H, Che C. Studies on Di- and Triterpenoids from *Salvia staminea* with Cytotoxic Activity. Planta Med. 2003; 69:464-467.
32. Gianfranco F, Giuseppe S, Benjamın R, Christina M, Dersch C, Richard B, *et al*. Synthetic studies on neoclerodane diterpenes from Salvia splendens:oxidative modifications of ring A. Tetrahedron. 2009; 65:1708-1715.
33. Kawahara N, Inoue M, Kawai K. Diterpenoid from *Salvia greggii*. Phytochemistry. 2003; 63:859-862.
34. Jang S, Jeong S, Kim K, Kirn HJ, Yu HH, Park R, *et al*. Tanshinone IIA from *Salvia miltiorrhiza* Inhibits Inducible Nitric Oxide Synthase Expression and Production of TNF-α, IL-1β and IL-6 in Activated RAW 264.7 Cells. Planta Med. 2003; 69:1057-1059.
35. Esqueve B, Calderon J, Florez E, Sanhhez A. Abietane and icetexane diterpenoids from *Salvia ballotaeflora* and *Salvia axillaris*. Phytochemistry. 1997; 46 (3):531-534.
36. Jork H, Funk W, Fischer W, Wimmer H. Thin-Layer Chromatography Reagents and Detection Methods, 1994; Vol. 1a, VCH-Verlagsgesellschaft, Weinheim, Cambridge, New York.
37. Qian TX. Yan ZH, Li LN. Mono-Feruloyl-R, R-(+)-tartaric Acid form Salvia. J. Chin. Pharm. Sci. 1993; 2, 148-150.
38. Tanaka T, Nishimura A, Kouno I, Nonaka G, Young TJ. Isolation and Characterization of Yunnaneic Acids A–D, Four Novel Caffeic Acid Metabolites from *Salvia yunnanensis*. J. Nat. Prod. 1996; 59:843-849.
39. Tanaka T, Nishimura A, Kouno I, Nonaka G, Yang CR. Four New Caffeic Acid Metabolites, Yunnaneic Acids E-H, from Salvia yunnanensis. Chem. Pharm. Bull. 1997; 45:1596-1600.
40. Amanda JL, Graeme B, Wayne B, Bowler DF, Brenda W, Toshio K, *et al*. The Osteoclast-associated Protease Cathepsin K Is Expressed in Human Breast Carcinoma. Cancer Res. 1997; *57*:5386-5390.

Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata* (Lour.) Stem Bark

Bhakta P Gaire[1*], Ramakanta Lamichhane[1], Chitra B Sunar[1], Amrita Shilpakar[1], Sabita Neupane[1], Sushil Panta[1]

[1]School of Health and Allied Sciences, Pokhara University, P. O. Box 427, Lekhnath – 12, Dhungepatan, Kaski, NEPAL.

ABSTRACT

Background: Stem barks of *Ficus auriculata* have been used in Nepal as folk remedy in the form of juice to treat diarrhea, dysentery, cuts and wounds. So this study is designed to explore the antioxidant and antibacterial activity of *F. auriculata* stem bark. **Methods:** Stem barks were subjected to successive extraction using hexane, chloroform and methanol to obtain the respective extracts. DPPH free radical scavenging method was used for antioxidant activity analysis. Agar well diffusion method was used for the antibacterial activity test. **Results:** Qualitative phytochemical analysis of methanol extracts of *F. auriculata* stem bark showed the presence of alkaloids, carbohydrates, saponins, glycosides, phytosterols, resins, phenols, tannins, diterpenes, flavonoids, proteins, and amino acids. Antioxidant activity of methanol extract was found to be 84.088% at 0.1 mg/ml with IC_{50} value of 0.042 mg/ml ($r^2 = 0.9942$) and that of chloroform extract was found to be 83.864% with IC_{50} value of 0.029 mg/ml ($r^2 = 0.955$). However free radical scavenging activity of hexane extract was of 42% ($r^2 = 0.9036$) in comparison of standard ascorbic acid used. Antibacterial activity of methanol extract was higher for *Escherichia coli* with 4.5±0.15 mm zone of inhibition and for *Staphylococcus aureus* hexane extract was highly effective with the zone of inhibition of 7.8±0.36 mm. **Conclusion:** Plant extract showed potential antioxidant activity but antibacterial activity was found to be comparatively lower to that of the standard antibiotics used.

Key words: ***F****icus auriculata*, **p**hytochemical screening, **a**ntibacterial activity, **a**ntioxidant activity, **D**PPH

INTRODUCTION

Ficus, the fig genus, consists of over 800 species in 40 genera of the mulberry family, Moraceae. A number of *Ficus* species are used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine (TCM) to treat several common ailments. Thirty-six species of *Ficus* are reported so far from Nepal but the detail investigation of their indigenous uses have never undertaken till now.[1] *Ficus auriculata* Lour. is widely distributed in temperate, tropical and subtropical regions of about 1800 – 2600 m altitude.[2] It is tree of about 4 -10 m tall and dioecious in nature. It contains abundant amount of white latex in every part of the plant.[3] Bark is grayish brown with rough texture. Branchlets are reddish brown. Figs (also called as fruits) are reddish brown, pear-shaped, depressed globose or top-shaped in nature and generally occur on leafless branchlets at base of trunk and main branches.[4-5]

The ethnomedicinal and traditional uses of *F. auriculata* in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, jaundice[6] etc. suggest that the plant must have antimicrobial as well as antioxidant efficacy. Several studies on other species of *Ficus* have shown the potential antioxidant and antimicrobial activity, but clinical researches regarding these properties of *F. auriculata* have not been carried out. So our main aim in this research is to explore the antibacterial and antioxidant efficacy of different extracts of bark of *F. auriculata*. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.[7,8] That's why before investigating pharmacological activity of plant extracts preliminary phytochemical screening plays the pivotal role.

Antioxidant defense system combats against wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson,

***Address for correspondence:**
E-mail: samarpanbp@gmail.com

DOI: 10.5530/pj.2011.21.8

and coronary heart pathologies resulted due to the free radicals and oxidative stress.[9-11] The main cause of oxidative stress in the body includes free radicals namely, superoxide ($O_2^{-\cdot}$), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), peroxynitrite ($^{\cdot}ONOO^{\cdot}$) and nitric oxide ($NO^{\cdot}$) radicals as well as non free radical species as hydrogen peroxide (H_2O_2), nitrous acid (HNO_2) and hydrochlorous acid (HOCl).[12-13] Reactive oxygen species (ROS) can easily initiate the lipid peroxidation of the membrane lipids, causing damage of the cell membrane of phospholipids and lipoprotein by propagating a chain reaction cycle. Thus, antioxidants are coevolved with aerobic metabolism to counteract oxidative damage from ROS.[14-16]

Nowadays lots of synthetic antioxidants are subjected in Pharma markets by several manufacturers but these synthetic antioxidants possess greater risks of side effects; therefore, investigations on identifying the natural antioxidants have become very important issue.[17] In the past few years, natural antioxidants have generated considerable interest in preventive medicine. Plants produce a huge amount of antioxidants and they can represent a potential source of new compounds having antioxidant properties with fewer side effects.[18-19]

The screening of medicinal plants for active compounds has become very important because these may serve as promising sources of novel antibiotic prototypes. Contrary to the synthetic drugs, antibacterial activities of phytochemicals are associated with lesser side effects and have an enormous therapeutic potential to heal many infectious diseases.[20] The potential for developing antibacterial from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Nowadays a number of clinically efficacious antibiotics are becoming less effective due to development of resistance and this has caused serious clinical problems in the treatment of infectious diseases.[21-24] So, biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens.[25]

MATERIALS AND METHODS

Plant collection and authentication

The plant barks were collected from the forest of Lekhnath municipality of Kaski district of Nepal and properly identified and confirmed in the department of Pharmacognosy of Pokhara University, where voucher specimen were deposited with the number HN 1294. The crude stem bark sample was deposited into the crude drug preservation laboratory with SN 387. Bark were thoroughly cleaned with tap water, lichens on the outer surfaces were removed by scrapping with stainless still knife, cut into pieces of dimension about 1.5 – 2 × 1 × 0.5 cm^3 and shed dried till complete dryness [Figure 1].

Other materials in experiment

Gram positive, *Staphylococcus aureus*, and gram negative *Escherichia coli*, used in the study, were obtained from the microbiology department of WHO – GMP certified Nepal Pharmaceuticals Laboratory Pvt. Ltd, Birgunj, Nepal. All chemicals used in the research purposes were obtained from the Merck and Qualigens fine chemicals. They were of analytical grade.

Preparation of extract

4 kg of dried bark pieces were macerated using methanol at room temperature for 48 h. Then the extracts obtained were filtered to obtain methanol extract. Thus obtained methanolic extract was concentrated in rotary flash evaporator and dried in a vacuum oven so as to obtain thick, viscous mass. The concentrated methanolic extract was subjected to successive extraction using hexane, chloroform and methanol to obtain hexane, chloroform and methanol soluble fractions. Flow-chart of overall extraction process is shown in figure 2.

Phytochemical screening

Phytochemical screening was carried out for hexane, chloroform and methanol soluble fractions as per the standard

Figure 1: Stem bark pieces of *F. auriculata*.

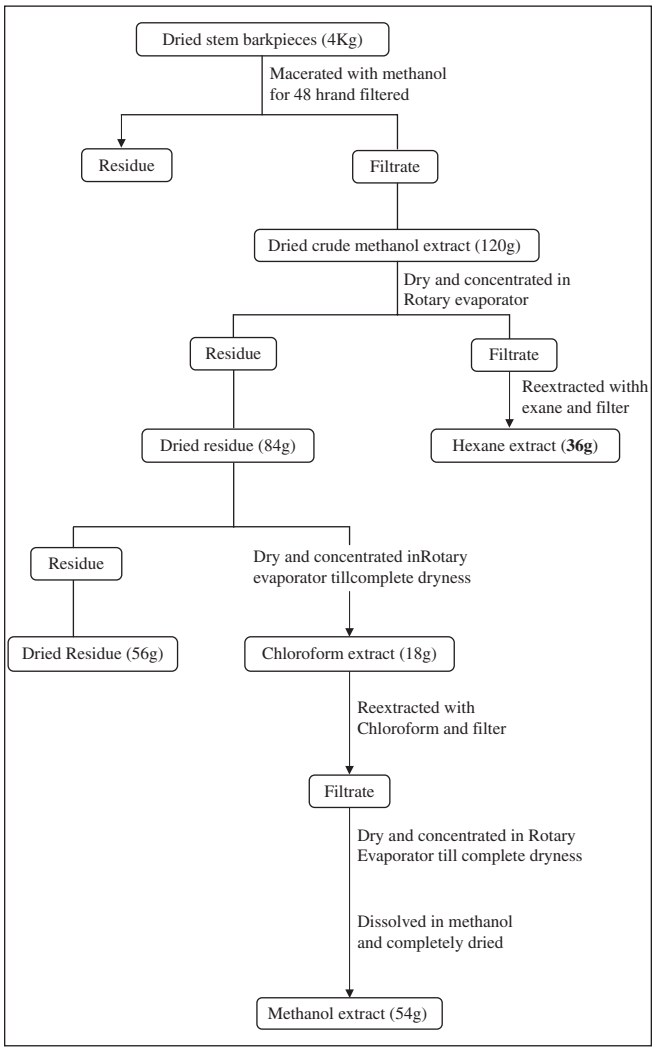


Figure 2: Flow chart of extraction process.

methods described[26-27] for the screening of alkaloids, carbohydrates, saponins, glycosides, flavonoids, resins, phenols, fats and fixed oils, phytosterols, tannins, diterpenes and proteins and amino acids. Each test was repeated at least three times to confirm the presence or absence of the phytochemicals. Reagents for phytochemical screening were freshly prepared according to the stated guidelines.[28-29]

Antioxidant activity test

Antioxidant activity was measured by DPPH free radical — scavenging method as described.[30] Solutions of concentration 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml were prepared for each extract in methanol. Freshly prepared 10 ml DPPH solution (1 mMol) was mixed with 20 ml of different samples (0.02 – 0.1) mg/ml. Ascorbic acid solution of same concentrations in methanol were prepared and used as positive control for the radical - scavenging activity test. Fifteen min later, the absorbance was measured at 517 nm. Each test was repeated a least three times for the reproducibility of the result.

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

$$\% \text{ free radical scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

Where,

Abs control = Absorbance of DPPH solution
Abs sample = Absorbance of extracts and ascorbic acid solutions[30-31]

Antibacterial activity

Each extract was dissolved in DMSO to produce solutions of concentration 20 mg/ml. Solutions of tetracycline and ofloxacin of 1 mg/ml concentration was made in DMSO for antibacterial assay. Each extract and antibiotic solutions are sterilized by filtration through a 0.22 µm membrane filter.[32]

Preparation of the culture media

Antimicrobial assay sensitivity medium (AASM) was used to prepare bacterial culture media for antibacterial sensitivity test. The culture media was prepared by dissolving AASM in distilled water. A turbid solution was obtained which was heated to clear transparent solution with continuous shaking to dissolve the agar completely. Then the solution was transferred to 21 test tubes so that each contained 25 ml of the solution. After that media was sterilized in autoclave at 121°C for 15 min at 15 lbs pressure. Sterilized media were then let to cool around 50°C. Then first 10 tubes were inoculated with bacterial suspension of *E. coli* and next 10 tubes with *Staphylococcus aureus* .One test tube media was used for negative control. The inoculated media were poured into the sterilized petriplates of 9 cm diameter and let to solidify. After solidification of the media, 5 bores were made in Petri plate separated at least by 2.54 cm from each other. 50 µl of each extract (20 mg/ml) were poured in three bores, solution of antibiotic in other bore and the solvent in the last bore. 2 plates were kept for positive control of both of the organism and 1 plate for negative control purposes. Plates were kept in freeze for 1 hr to let the proper diffusion of extract and antibiotic solution in the media. Then they were incubated in BOD incubator for 24 hr at 37°C. After 24 hr zone of inhibition was measured. Each test was repeated at least 3 times in the aseptic environment for the reproducibility of the result.[27,33]

Statistical analysis

Mean zone of inhibition was compared using ANOVA and the data were presented as mean ± SD. $p < 0.05$ was considered as significantly different while measuring the level of significance.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening of different extracts are shown in [Table 1]. Phytochemical screening revealed the presence of carbohydrates, alkaloids, saponins, resins, phenols, proteins and amino acids in all three extracts. Phytosterols and flavonoids were present in chloroform and methanol extracts but were absent in hexane extracts. Glycosides were found to be present only in methanol extracts. Fats and fixed oils were not present in any of the extracts.

Antioxidant (free radical scavenging) activity

The free radical scavenging activity of DPPH free radical is shown in [Figure 3]. In comparison to the standard ascorbic acid, both of chloroform (IC_{50} = 0.029 mg/ml) and methanol (IC_{50} = 0.042 mg/ml) extracts showed more than 85% of free radical scavenging efficacy [Figure 4]; whereas hexane soluble fraction showed least antioxidant efficacy of about 42%. Typical phenolic compounds that possess antioxidant activity have been characterized as phenolic acids and flavonoids.[34] In the present study the antioxidant activity test was found to be positive for each extract which can be attributed to the presence of phenols and flavonoids as shown by the phytochemical screening test.[35] The comparative study showed the higher antioxidant activity of methanol and chloroform extract than that of hexane extract. The methanol and chloroform extract showed the presence of both flavonoids and phenols while hexane extract contained only phenol. The presence of both types of antioxidant, flavonoids and phenolic compounds in chloroform and methanol extracts can be concluded for higher antioxidant activity of these extracts as compared to hexane extract.

Table 1: Results of phytochemical screening of different extracts.
Among tested phytochemicals, alkaloids, saponins, carbohydrates, phenols and protein and amino acids are present in all three extracts.

Phytochemical Test	Reagents used (test performed)	Interference for presence of phytochemicals	Results		
			Hexane extract	Chloroform extract	Methanol extract
Alkaloid test	Mayer's Reagents	Appearance of yellow cream ppt	Presence	Presence	Presence
	Hager's Reagents	Formation of yellowish white ppt			
	Hager's Reagents	Formation of yellow color ppt			
Carbohydrate test	Molish's reagent	Formation of violet ring	Presence	Presence	Presence
	Benedict's reagent	Formation of orange red ppt			
	Fehling's reagent	Formation of red ppt			
Saponins test	Foam Tests	Produce foam that lasts for more than 10 min	Presence	Presence	Presence
Glycoside test	Modified Brontrager's reagent	Formation of rose pink color	Absence	Absence	Presence
	Legal's reagent	Formation of pink/blood red color			
Phytosterols test	Salkowski's Test	Golden brown color obtained	Absence	Presence	Presence
Fats and Fixed oil test	Filter Paper press Test	Oily stain was obtained	Absence	Absence	Absence
Resin test	Acetone Water Test	Appearance of turbidity	Absence	Presence	Presence
Phenol test	Ferric Chloride Test	Appearance of bluish black ppt	Presence	Presence	Presence
Tannin test	Gelatin Test	Formation of white ppt	Presence	Absence	Presence
Diterpenes test	Copper Acetate Test	Formation of bright green color	Absence	Presence	Presence
Flavonoids test	Alkaline Reagent Test	Intense yellow color obtained	Absence	Presence	Presence
	Lead Acetate Test	Yellow ppt obtained			
	Shinoda Test	Formation of magenta color			
	Zinc HCl Test	Formation of magenta color			
Proteins and amino acids test	Xanthoproteic Test	Formation of yellow color	Presence	Presence	Presence

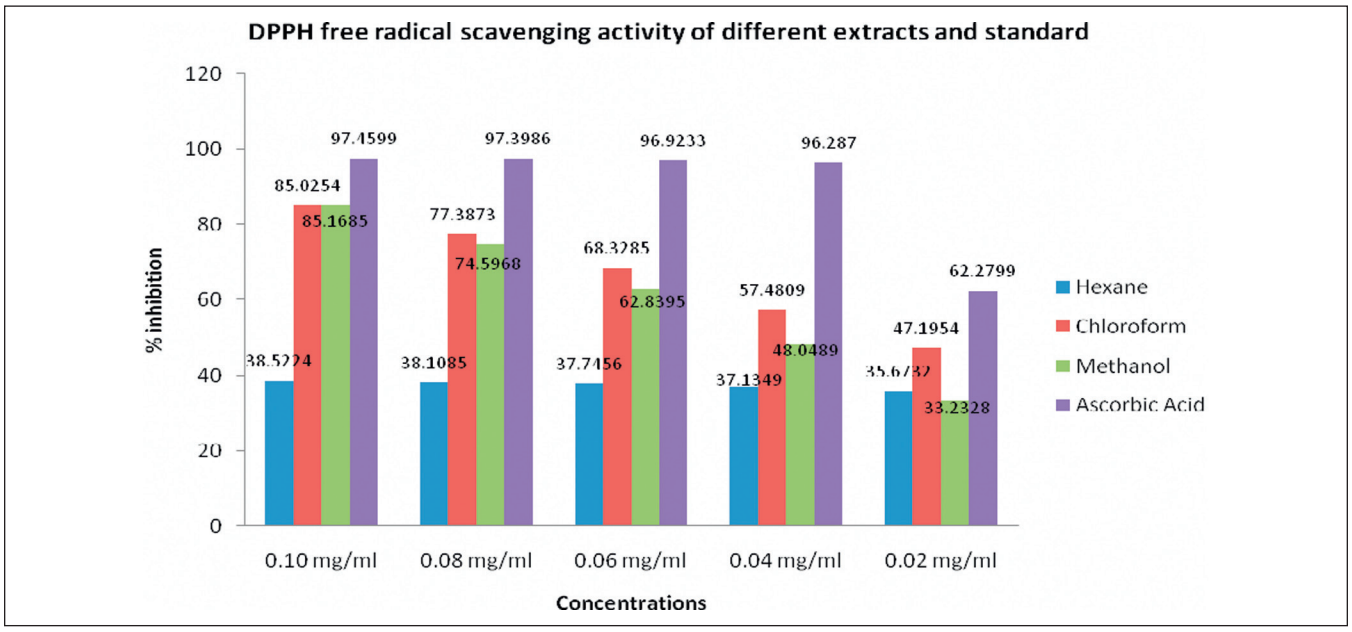


Figure 3: DPPH free radical scavenging activity of different extracts and standard at different concentrations.

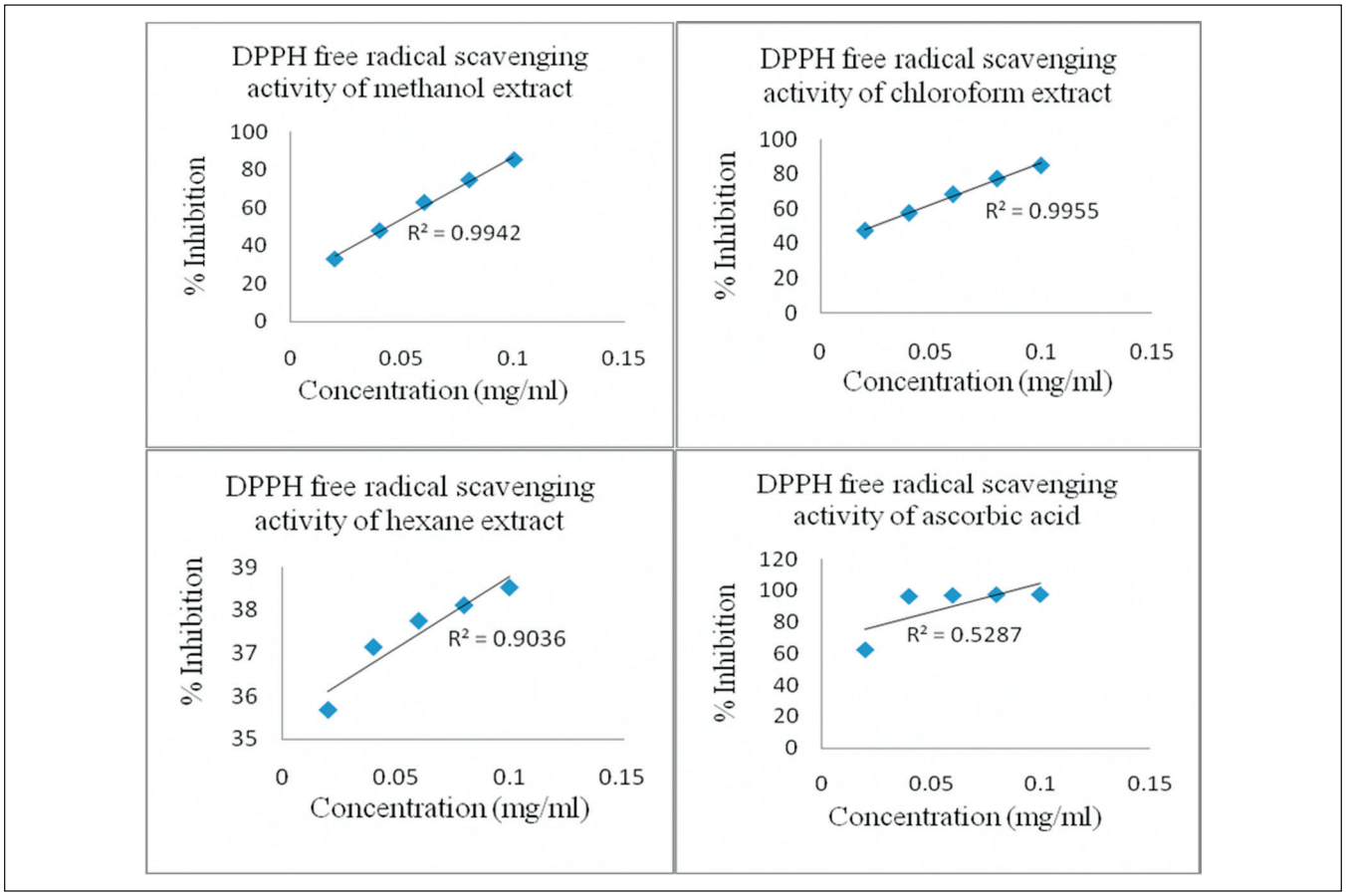


Figure 4: Regression coefficient measurement to measure the linearity of the curve.

Antibacterial activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants.[33,36] The antimicrobial activity of extracts of *F. auriculata* was tested under *in vitro* conditions

Table 2: Mean diameter of zone of inhibition by extracts and standards.
Methanolic extracts shows the higher antibacterial effect for *E. coli* whereas hexane extract shows the higher antibacterial activity for *S. aureus* ($p < 0.02$).

Selected organism	Zone of Inhibition in mm (Mean ± standard deviation)				
	Hexane Extract	Chloroform Extract	Methanolic Extract	Ofloxacin Standard	Tetracycline Standard
Escherichia coli	3.2 ± 0.09	1.4 ± 0.03	4.5 ± 0.15	11.5 ± 0.15	10 ± 0.03
Staphylococcus aureous	7.8 ± 0.36	7.3 ± 0.21	6.9 ± 0.03	34.3 ± 0.06	32 ± 0.03

by agar well diffusion. Antibacterial activity of hexane, chloroform and methanol extract were evaluated against two bacterial species *E. coli* and *S. aureus* which are known to cause diarrhea dysentery, abscesses, wounds and other infections in human. To evaluate the antibacterial efficacy of different extracts against standard antibiotics; zone of inhibition [Table 2] were measured. Methanolic extract showed greater zone of inhibition for *E. coli* than that of hexane extract, whereas chloroform extract showed the least antibacterial effect. In case of *S. aureus*, hexane extract showed greater zone of inhibition than that of chloroform extract whereas methanolic extract showed least antibacterial activity. Tannins are considered as the potential antimicrobials generally present in the plant bark. Both hexane and methanol extract showed the presence of tannins in preliminary phytochemical screening. So antibacterial activity of *F. auriculata* is may be due to the presence of tannins constituents of bark.[37] Among standard antibiotics used ofloxacin showed greater zone of inhibition for both *E. coli* and *S. aureus*, than that of tetracycline. Antibacterial test confirmed that all of the extracts possess antibacterial property. These findings support the traditional uses of the plant to treat cuts, wounds, diarrhea, dysentery, etc.

CONCLUSION

This study has explored the various phytochemicals, including glycosides, phenols and flavonoids, present in the bark of *F. auriculata* for the first time. The Antioxidant efficacy of methanol and chloroform extract, being equivalent to standard ascorbic acid at concentration of 0.1 mg/ml, indicates that this plant can has great scope for isolation and identification of important antioxidant molecules which can be formulated to make antioxidant dosage forms. On top of that, these natural antioxidants can have potential advantages among various diseases with oxidative stress. This plant also showed promising antibacterial activity even though it was comparatively weaker than standard antibiotics. If we can purify and isolate the antibacterial Biomolecules then definitely they would give equivalent antibacterial activity to that of standard antibiotics. So, further study is necessary to get maximum benefit from this plant. It can play a pivotal role to change the traditional system of medicine into a scientific and standard medication system. So this study confirming the presence of antioxidant and antibacterial activity of this plant has indicated the further necessary study on this plant for isolation and identification phytochemicals of such property.

ACKNOWLEDGEMENTS

We sincerely thank to Prof. Dr. Purusotam Basnet, The National Research Centre in Complementary and Alternative Medicine (NAFKAM), Faculty of Health Sciences Tromsø University, Norway; for reviewing this manuscript. We wish to express our gratitude to Dr. Nirmala Jamarkattel, Mr. Namraj Dhami, and School of Health and Allied Sciences family for their kind support.

REFERENCES

1. Kunwar RM, Bussmann RW. *Ficus* (Fig) species in Nepal: a review of diversity and indigenous uses. J Eco App. 2006; 11:85-97.
2. Kala CP. Prioritization of cultivated and wild edibles by local people in the Uttaranchal hill of Indian Himalaya. Indian J Trad Know. 2007; 6:239-44.
3. Weiblen GD. Phylogenetic relationship of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. Am J Bot. 2000; 87:1342-57.
4. Wang HK, Hyde KD, Soytong K, Lin FC. Fungal diversity on fallen leaves of *Ficus* in northern Thailand. J Zhejiang Univ Sci B. 2008; 9:835-41.
5. Schulz JB, Lindnau J, Seyfriend J, Dichgans J. Glutathione oxidative stress and neuro-degeneration. Eur J Biochem. 2000; 267:4904-11.
6. Gairola Y, Biswas S. Bioprospecting in Garhwal Himalayas, Uttarakhand. Current Sci. 2008; 94:1139-44.
7. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula, Terminalia belerica and Emblica officinalis*. BMC Complement Altern Med. 2010; 10:20.
8. Radhika B, Nasreen Begum, Srisailam K. Pharmacognostic and Preliminary Phytochemical Evaluation of the leaves of *Bixa orellana.* Phcog Net. 2010; 2:132-6. |
9. Veerapur VP, Prabhakar KR, Parihar VK, Kandadi MR Ramakrishana S, Mishra B, Rao S, Satish BS, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK. *Ficus racemosa* Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector. eCAM. 2009; 6:317-24.
10. Lu JM, Lin PH, Yao Q, Chen C. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. J Cell Mol Med. 2010; 14:840-60.
11. Sánchez JC, García RF, Cors MM. 1,1-Diphenyl-2-picrylhydrazyl radical and superoxide anion scavenging activity of *Rhizophora mangle* (L.) bark. Phcog Res. 2010; 2:279-84
12. Manandhar NP. Plants and People of Nepal, Timber Press, 2002.
13. Mavi A, Terzi Z, Ozgen U, Yildirim A, Coskun M. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (apiaceae), *Sedum*

sempervivoides (crassulaceae) *Malva neglecta* (malvaceae), *Cruciata taurica* (rubiaceae), *Rosa pimpinellifolia* (rosaceae), *Galium verum* subspecies *verum* (rubiaceae), *Urtica dioica* (urticaceae). Biol Pharm Bull. 2004; 27:702-5.

14. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol. 2002; 79:379-81.
15. Maxwell SR. Prospects for the use of antioxidant therapies. Drugs. 1995; 49:345-61.
16. Schinor EC, Salvador MJ, Ito IY, Dias DA. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. Braz J Microbiol. 2007; 38:145-9.
17. Koduru S, Grierson DS, Afolayan AJ. Antimicrobial activity of *Solanum aculeastrum*. Pharm Biol. 2006; 44:283-6.
18. De Lencastre H, Oliveira D, Tomasz A. Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. Curr Opin Microbiol. 2007; 10:428-35.
19. Mishra NN, Prasad T, Sharma N, Paysi A, Prasad R, Gupta DK, Singh R. Pathogenicity and drug resistance in *Candida albicans* and other yeast species. A review. Acta Microbiol Immunol Hung. 2007; 54:201-35.
20. Torres JA, Villegas MV, Quinn JP. Current concepts in antibiotic resistant Gram negative bacteria. Expert Rev Anti Infect Ther. 2007; 5:833-43.
21. Bastos AM, Lima FM, Conserva LM, Andrade VS, Rocha EM, Lemos RP. Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Ann Clin Microbiol Antimicrob. 2009; 8:16.
22. Mosquera OM, Correa YM, Buitrago DC, Nifno J. Antioxidant activity of twenty five plants from Colombian biodiversity. Mem Inst Oswaldo Cruz. 2007; 102:631-4.
23. Usman H, Abdulrahman FI, Usman A. Qualitative phytochemical screening and in vitro antimicrobial effects of Methanol stem bark extract of *Ficus thonningii* (moraceae). Afr J Trad CAM. 2009; 6:289-95.
24. Vishnoi NK. Advanced Practical Organic Chemistry. Vikash Publication House Private Limited 2000.
25. Kumaraswamy MV, Kavitha HU, Satish S. Antibacterial evaluation and phytochemical analysis of *Betula utilis*. World Journal of Agricultural Sciences. 2008; 4:661-4.
26. Roopashree TS, Raman D, Rani SRH, Narendra C. Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia and Calendula officinalis*. International Journal of Applied Research in Natural Products. 2008; 1:20-8.
27. Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HB. Phytochemical and antioxidant screening of some plants of Apocynaceae from south west Nigeria. African Journal of Plant Science. 2008; 2:124-8.
28. Paul SB, Mazumder AH, Gogoi HK, Gogoi BJ, Chaurasia AK, Singh L, Srivastava RB. Evaluation of *In vitro* Antioxidant activity of some plants of Cachar District, Assam. Phcog Net. 2010; 2:289-92.
29. Priya P, Pal JA, Aditya G, Gopal R. Anti-microbial, Anti-oxidant and Anthelmintic Activity of Crude Extract of *Solanum xanthocarpum* Pharmacognosy J. 2010; 2:400-4.
30. Tung YT, Wu JH, Kuo YH, Chang ST. Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. Bioresour Technol. 2007; 98:1120-23.
31. Mishra AK, Sahu N, Mishra A, Ghosh AK, Jha S, Chattopadhyay P. Phytochemical screening and antioxidant activity of essential oil of Eucalyptus leaf. Pharmacognosy J. 2010; 2:21-24.
32. Shao HB, Chu LY, Lu ZH, Kang CM. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biol Sci. 2008; 4:8-14.
33. Mothana RA, Lindequist U, Gruenert R, Bednarski PJ. Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the Island Soqotra. BMC Complemet Altern Med. 2009; 9:1-11.
34. Alma MH, Mavi A, Yildrim A, Digrak M, Hirata T. Screening chemical composition and in vitro antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. Biol Pharm Bull. 2003, 26:1725-9.
35. Govindarajan R, Rastogi S, Vijayakumar M, Shriwaikar A, Rawat AKS, Mehrotra S et al. Studies on the antioxidant activities of *Desmodium gangeticum*. Biol Pharm Bull. 2003; 26:1424-7.
36. Behera SK, Misra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. J Ethnopharmacol. 2005; 102:319-32.
37. Singh M, Khatoon S, Singh S, Kumar V, Rawat AK, Mehrotra S. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Phcog Res 2010; 2:254-7.

Evaluation of Antiobesity Activity of Various Plant Extracts

D'Mello P. M[1], Darji K.K[1], Shetgiri P.P[1]

[1]*Department of Pharmacognosy and Phytochemistry, Prin. K.M. Kundanani college of Pharmacy, Cuffe parade, Colaba, Mumbai-400005, India.*

ABSTRACT

Obesity is one of the major factors contributing to increase prevalence of various pathophysiological disorders like hyperlipidemia, diabetes mellitus and congestive heart diseases. Attempts made to reduce body fat can be positively linked with lower risk involved in these diseases. The present paper emphasizes on study related to the antiobesity effect of plant extracts in Albino Wiatar rats fed with high atherogenic diets. The effect of plants extract like *Vitis vinifera*, *Oroxylum indicum, Garcinia indica* and a phytoconstituent isolated from sugarcane wax i.e. polycosinol were evaluated on parameters like body weight, organ weight and biochemical parameters like serum glucose. The plant extracts exhibited good antiobesity effect when compared with the diseased state group fed with high cholesterol rich diet. The blood sugar level and body organ weights were also found to be reduced significantly as compared to normal group. All these extracts are rich in polyphenols which may be responsible for their antiobesity activity.

Key words: Obesity, Polycosinol, *Vitis vinifera*, *Oroxylum indicum, Garcinia indica.*

INTRODUCTION

Obesity is a serious health problem worldwide leading to several pathophysiological disorders like hyperlipidemia, diabetes mellitus and congestive heart diseases. Among the multiple factors contributing to its etiology are the sedentary life style, lack of exercise, endocrine disorders, physiological and psychological factors, smoking and pregnancy at a later age, certain drugs that increase body weight, mental illness and the consumption of energy rich junk foods.[1,2] Obesity increases the mechanical and metabolic load on the myocardium, thus increasing myocardial oxygen consumption.[3] Increased fat deposition may be a result of the predominant consumption of hyperlipidemic diets. The primary treatment for obesity is dieting and physical exercise. To supplement this, or in case of failure, anti-obesity drugs may be taken to reduce appetite or inhibit fat absorption. Also the non availability of drugs for its treatment is a major concern as drugs that reduce obesity can also have implications towards liver, heart and spleen.[4,5] Natural products identified from traditional medicinal plants have always presented an exciting opportunity for the development of newer therapeutic agents. The objective of the present study was to evaluate antiobesity effect of various plant extracts like *Vitis vinifera*, *Oroxylum indicum, Garcinia indica* and a phytoconstituent isolated from sugarcane wax i.e. polycosinol by artherogenic diet induced obesity model in Albino Wistar Rats.[6,7]

Address for correspondence:
Fax no: 91-02222165282 ; 91-022 22164368
E-mail: dmellopm@rediffmail.com

DOI: 10.5530/pj.2011.21.9

MATERIAL AND METHODS

Collection of plant material and Extraction: *Garcinia indica* fresh fruits were collected from forest of Ratnagiri District of Maharashtra, India and authenticated by Dr.A.M.Majumdar from Agharkar Research Institute, Pune, Maharashtra. The fruits were cut into two pieces and sun dried. The dried material was powdered and was subjected to soxhlet extraction using methanol as a solvent. *Vitis Vinifera* commonly known as black Grapes were obtained from local sources, the fruits were dried and subjected to soxhlet extraction using methanol as a solvent. *Oroxylum indicum* was obtained from local sources and authenenticated by Dr. Naik at Nicholas Piramal Laboratories Ltd and subjected to soxhlet extraction using methanol as a solvent. Policosanol was isolated from sugar cane wax by known methods.[8] Methanol was evaporated and the extracts reconstituted in water were used for the study.

Experimental Animals: For antiobesity study Albino Wistar Rats (120-150 gm) were obtained from Glenmark

pharmaceuticals ltd., Mumbai. All the animals were maintained under good hygienic conditions in the animal house of Prin. K.M. Kundnani College of pharmacy. Animals were maintained under standard environmental conditions (22-28°C, 60-70% relative humidity, 12 hr L: D cycle) and fed with standard diet and water *ad libitum*. The experimental protocol was approved by the institutional animal ethics committee. Animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days period prior to experimental study. The experimental work was carried out as per Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidlines.

Parameters tested and procedures

Body weight: The body weight (g) was recorded from day 1 twice a day before administration of drug for 22 days in each group.

Locomotor activity: It was observed every day before administration of plant extracts visually by placing the rat in the center table and recording the physical ambulatory changes.

Biochemical parameters: At the end of 22 days blood sugar levels was measured from serum samples using the biochemical kits.

Organ weights: The animals were sacrificed by cervical dislocation and then liver, heart, were removed and weighed.

Evaluation of antiobesity activity

Albino wistar female rats (120-150 gm) were divided into ten different groups each containing 6 animals in each which are as follows:

Group 1 Control group:	Only vehicle was given to this group.
Group 2 Hyperlipidemic group:	Cholesterol in coconut oil (25 mg/kg/ day) was administered to each rats of this group.
Group 3 Test group:	Rats of this group were administered with *Garcinia indica* extract (400 mg/kg/ day) along with cholesterol in oil.
Group 4 Test group:	Rats of this group were administered with *Policosanol* (200 mg/kg/ day) along with cholesterol in oil.
Group 5 Test group:	Rats of this group were administered with *Vitis vinifera* extract (200 mg/ kg/day) along with cholesterol in oil.
Group 6 Test group:	Rats of this group were administered with *Oroxylum indicum* extract (200 mg/kg/day) along with cholesterol in oil.

The cholesterol in oil was given to each animal except the control group daily at 10.00 am.[9,10] The solution of extract was prepared freshly every day and given to their respective group at 3.00 pm. This process was followed for 22 days and the amount of food intake was monitored daily. At the end of experimental studies animals were fasted for 12 hr and then the blood was collected by cardiac puncture under light ether anesthesia animals were then sacrificed by ether anesthesia. The liver was isolated and preserved in 10% formalin solution.[11] The biochemical parameters were evaluated using diagnostic kits. All the results were subjected to statistical analysis using one way Anova followed by Dunnett's multiple comparison tests against the hyperlipidemic group. The p values < 0.05 were considered significant. All the values were expressed as mean ± S.E.M and compared with control group.

RESULTS

The evaluation of antiobesity activity using cholesterol induced hyperlipidemia shows that the groups treated with plant extracts had a significant antiobesity activity. The significant reduction in the increments in body weights of the rats treated with plant extract was observed when compared with the hyperlipidemic group (Figure 1). The groups treated with plants extract had significant reduction in the levels of blood sugar than the toxicant group (Table 1). The organ weight increment is also significantly reduced in the test groups as compared to toxicant group. Animals did not posses any significant changes in ambulatory activity as compared to control group.

DISCUSSION

The groups treated with plants extract exhibited significant antiobesity activity. All this palnt extracts are rich in polyphenol content which may be responsible for their significant antiobesity activity. *Garcinia indica* is known to contain phenolic phytochemicals like garcinol which is polyisoprenylated benzophenone and hydroxy citric acid (HCA).[12,13] Policosanol was isolated from sugarcane wax which also exhibited antiobesity activity. *Vitis vinifera* is another such plant rich in flavonoids like quercetin, anthocyanins, carotenes, ascorbic acid etc while *Oroxylum*

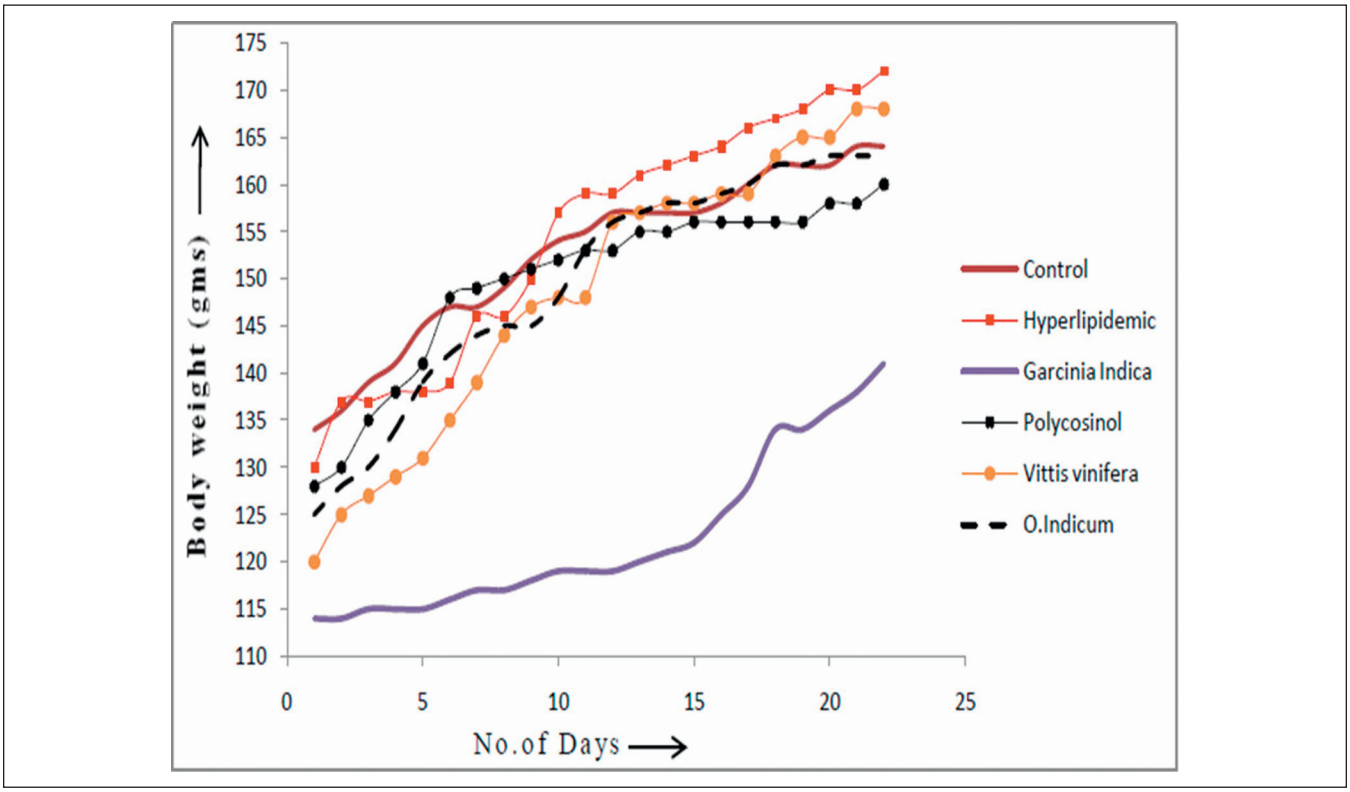


Figure 1: Graph depicting antiobesity effects of various plant extracts

Table 1: Effect of plant extracts on Blood sugar and organ weight

Groups	Blood sugar (mg/dl)	Organ weight (Liver in Gm)
Control	96.6	5.8
Hyperlipidemic	122.4	7.2
Garcinia indica	93.5	5.9
Policosanol	97.9	6.2
V. vinifera	103.5	6.4
O. indicum	94.7	6.1

indicum is rich in flavones.[14,15] In toxicant group animals, there was significant increase in weight of liver as compared to treatment with plant extracts which indicate the fat deposition in the hyperlipidemic group leading to obesity. The least organ weight was observed in the group treated with *Garcinia indica* which has the maximum antiobesity activity. Earlier researchers have reported the role of hydroxy citric acid as an appetite suppressant which is one of the constituents of the extract of *Garcinia indica* .[16] The activity also could be the result of synergism between several constituents like garcinol, anthocyanins along with hydroxyl citric acid present in the extract. Plant extracts treatment group significantly reduced the blood glucose level when compared with toxicant group fed with high cholesterol.

These findings suggest that extracts supplements reduce the obesity caused by high fat diet and normalize body weight. Whilst orthodox medicine concedes, in fact insists, that further research is necessary, the implications of these findings are exciting; suggesting that there may be many more as yet undiscovered benefits of flavonoids. As always, however, the holistic functioning of the body means that maximum benefits will only be obtained by the consumption of the widest possible variety of all these compounds.

REFRENCES

1. Caterson ID. Obesity and its management. Australian Prescriber, 1999; 12:6-12.
2. Rippe J., Crossley S., Ringer R. Obesity as a chronic disease: Modern medical and lifestyle management. J Am Diet Assoc., 1998; 98:9-15.
3. Dietz W., Goodwin N., Yanovski S. Long-term pharmacotherapy in the management of obesity. JAMA .1996; 276:1907-15.
4. Vincent H, Powers S, Dirks A, Scarpace P. Mechanism for obesity-induced increase in myocardial lipid peroxidation. Int J Obes (Lond) 2001; 25:378-388.
5. Lemhadri A., Eddouks M., Sulpice T. Remy Burcelin. Anti-hyperglycaemic and Anti-obesity Effects of *Capparis spinosa* and *Chamaemelum nobile* Aqueous Extracts in HFD Mice. American Journal of Pharmacology and Toxicology 2007; 2 (3):106-110
6. Kaur G and Kulkarni S. Anti obesity effect of a polyherbal formulation OB-200 g in female rats fed on cafeteria and artherogenic diets. Indian Journal of Pharmacology, 2000; 32:294-299.
7. Jian-Gao Fan. Effects of low-calorie diet on steatohepatitis in rats with obesity and hyperlipidemia. World J Gastroenterol 2003; 9 (9):2045-2049
8. Gu Q, Luo H., Zheg W., Huang Y. Int. J. Syst. Evol.Microbiol. 2006; 56:2193-97.

9. Dhuley J., Naik S., Rele S., Benarji A. Hypolipidaemic and antioxidant activity of diallyl disulphide in rats, Pharm. Pharmacol. commun. 1999; 5:689.
10. Ochani P. and D'Mello P. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* linn.leaves and calyces extract in rats, Indian J of experimental biology. 2009; 47:276-282.
11. Wiliamson E., Okpako M. Evans F. Pharmacological methods in phytotherapy research, (1st edn) Wiley and sons publication USA. 1996.
12. Jena B., Jayaprakasha G., Singh R. Sakariah K ,Chemistry and biochemistry of – hydroxycitric acid from *garcinia*, J. Agric. Food Chem. 2002; 50:10–22.
13. Selvi A, Joseph G. Jayaprakasha G. Inhibition of growth and aflatoxin production in aspergillus flavus by *garcinia indic*a extract and its antioxidant activity, J of Food Microbiology. 2003; 20:455-60.
14. Hmamouchi M. Flavones and flavanols in leaves of *vitis vinifera*. Fitoterapia, 1996; 68:113-114.
15. Mariangeli C, Kasssis N., Jain D, Ebine N and Jones P. Comparison of composition and absorption of sugarcane policosanols PJH. 2007; 97:381-388.
16. A.Mishra, M.Bapat and J Tilak,. Antioxidant activity of *Garcinia indica* (kokam) and its syrup, Current Science. 2006; 91:90-93.

Role of *Melia azedarach* leaf extract in Paracetamol Induced Hepatic damage in rats

Mohammed Fazil Ahmed*[1], A. Srinivasa Rao[2], Hameed Thayyil[1], Shaik Rasheed Ahemad[1] and Mohammed Ibrahim[1]

[1]Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near Ramoji Film City, Nalgonda,(AP), INDIA-508284.
[2]Bhaskar Pharmacy College,Yeknapally, Moinabad(Mandal), R.R(Dist), Hyderabad-500075.

ABSTRACT

The hepataprotective activity of the methanolic leaf extract of *Melia azedarach* was investigated against paracetamol induced hepatic damage in rats. Over dosing of paracetamol to rats is reported to decrease the activity of antioxidative enzymes (GPx, GST, SOD and CAT) in liver and increase the serum enzymes (SGOT, SGPT and Alkaline phosphate), bilirubin and decrease the total proteins content. *Melia azedarach* leaf extract maintained the activity of antioxidant enzymes and as well as serum enzymes to the normal level. Thus the present study ascertains that the leaf extract of *Melia azedarach* possesses significant hepatoprotective activity.

Key words: Hepatoprotective, Hepatic damage, Methanolic leaf extract, *Melia azedarach,* Serum enzymes and Antioxidant enzymes

INTRODUCTION

Liver diseases, especially viral hepatitis occur predominately in the developing world[1] with an enormous impact on public health and economy. Plant drugs in Indian ayurvedic system and Chinese herbal medicine have long been used for liver and bilary diseases. Some plants have also been found to possess hepatoprotective activity and the underlying mechanism of action involves their anti-oxidant property.[2-7] The oxidative stress is not only the causative of the liver damage but also implicated in the pathogeneses of cancer, diabetes, cardiovascular disorders as well as in the process of aging.[8] It is established that diabetes is associated with lower level anti-oxidants and many plants show hypoglycemic activity due to their anti-oxidant potentials.[9]

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes.[10] The hepatoprotective activity of the leaf extract of *Alchornea cordifolia* (Schum and Thonn), a Nigerian plant on acetaminophen induced toxicity *in vivo* has been reported.[11] The antioxidative properties revealed total phenolic content of 0.22 mg/ml and reducing power of 0.062 mg/ml as compared to vitamin E with a reducing power of 0.042 mg/ml. The authors concluded that the hepatoprotective activity of this plant on acetaminophen induced liver damage is connected to its antioxidative properties. As the plant under consideration is reported to have hypoglycemic activity it may also be useful in liver damage thus the present study was directed to investigate the hepatoprotective activity against paracetamol intoxicated rats.

Among generally used NSAIDS the frequently used is paracetamol, which is widely used analgesic antipyretic agent which is metabolized by the liver.[12] Overdosing of paracetamol to rats is reported to decrease their sensitivity to its hepatotoxic effect, which are associated with oxidative stress.[13] So this study on antioxidant enzymes glutathione peroxidase (GPx) glutathione-s-transferase(GST), super oxide dismutase (SOD) and catalase(CAT) have been found to be great importance in the assessment of liver damage.

Melia azedarach linn (meliaceae; Neem) is an indigenous plant possessing several medicinal properties. *Melia azedarach linn* (synonym: *Melia dubia* Cav, Indian lilac, Persian lilac) belonging to the family *Meliaceae* is a tree found in India. It is popular as Indian lilac. Different phytochemicals present in leaf, root and stem, are meliacarpins, limonoids, sendanins, trichilins and azedarachins.[14] The plant is traditionally used

***[1]Address for correspondence:**
E-mail: mohdfazil_pharma@yahoo.co.in

DOI: 10.5530/pj.2011.21.10

for the treatment of leprosy, inflammations, Analgesics and cardiac disorders. Its fruits extracts possess ovicidal[15] and larvicidal activity.[16] The leafs extracts also possess antiviral[17] and antifertility activity.[18]

MATERIALS AND METHOD

Plant materials

The leaves of *Melia azedarach linn* were collected from botanical garden, Annamalai University, Annamalainagar, Chidambaram-608 002. The plant was identified and authenticated by Department of Botany, Research Officer (botanist) Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India.

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material was extracted with methanol using soxhlet apparatus. The methanol extract was concentrated in vacuo and kept in a vacuum dessicator for complete removal of solvent. The yield was 9.1% w/w with respect to dried powder. Preliminary qualitative analysis of the methanol extract showed presence of alkaloids, tannins, glycosides and saponins. Now the methanolic extract is reading for present hepataprotective study.

Experimental Animals

The approval of institute animal ethics committee ware obtained from the animal house of Nizam Institute of Pharmacy, Deshmukhi, Ramoji film city, Hyderabad. Male albino Wistar rats 30 (100-150 g, 4-6 weeks old) was maintained under controlled conditions of light (12/24) and temperature (23 ± 1°C). Food pellets and tap water were provided ad libitum. For experimental purpose, animals were fasted overnight but were allowed free access to water. All animal experiment were carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee) with registration number. (1330/AC/10/CPCSEA)

Acute toxicity study

Melia azedarach in the dose range of 110 mg-630 mg/kg were administered orally to different group of mice comprising of ten mice in each group. Mortality was observed after 72 h. Acute toxicity was determined according to the method of Litchfield and Wilcoxon.[19]

METHODOLOGY

Five groups of rats, six in each received the following treatment schedule.

Group I: Normal control (saline)
Group II: Paracetamol treated control (2 g/kg.p.o)
Group III: Paracetamol (2 g/kg.p.o) + *Melia azedarach* leaf extract (500 mg/kg, p.o)
Group IV: *Melia azedarach* leaf extract (500 mg/kg, p.o)
Group V: Silymarin (25 mg/kg, p.o)

Leaf extract, paracetamol standard drug and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline. Group II received paracetamol (2 g/kg) for 7 days. A fixed dose of the leaf extract (500 mg/kg p.o) dose was given once daily to Group III animals for 7 consecutive days and paracetamol (2 g/kg, p.o) was administered on 5th day of the extract administration. Group IV & V received leaf extract (500 mg/kg, p.o) and Silymarin (25 mg/kg.p.o) for 7 days.

After 48 hours of last dose of paracetamol blood sample was collected from retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 rpm at 37°C and used for the assay of biochemical marker enzymes (SGOT, SGPT, and alkaline phosphatase), bilirubin and total protein. Immediately after collecting blood, the animals were sacrificed and livers dissected out for biochemical studies. SGOT, SGPT, Serum alkaline phosphatase (ALP) and bilirubin were determined by using commercially available kits (Span Diagnostic Ltd., Surat, India). Serum total protein was measured according to the method of Lowry et al, 1951.[20] Then the rats were sacrificed by cervical dislocation and liver removed immediately and transferred to ice cold container, homogenized and assayed for antioxidant enzymes such as glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase activity study using standard methods.[21-24]

Statistical Analysis

The data are represented as mean ± S.E.M. Student's t-test is used for statistical analysis of blood serum parameters (Table 1) and one-way ANOVA followed by Tukey kramer post test was used for statistical analysis of liver enzymes (Table 2). $p < 0.05$ was considered significant.

RESULTS

The acute oral toxicity study of *Melia azedarach* showed no mortality upto 610 mg/kg. The effect of methanol extract of *Melia azedarach* an serum transaminases, alkaline phosphates, bilirubin and total protein level in paracetamol intoxicated rats are summarized in Table 1. There was a significant ($p < 0.001$) increase in serum GOT, GPT, ALP, and bilirubin levels in paracetamol-intoxicated group compared to the normal control group.

The total protein levels were significantly ($p < 0.001$) decreased to 4.98 g/dl in paracetamol-intoxicated rats from the level of 6.85 g/dl in normal group. On the other hand the group with received both leaf extract and paracetamol (Group III) and extract alone (Group IV) showed significantly decreased the elevated serum marker enzymes when given orally and reversed the altered total protein to almost normal level (Table 1). The leaf extract and paracetamol and extract alone treated groups also reduced the level of bilirubin to 1.45 and 1.01 mg/dl respectively from the level of 1.98 mg/dl in the untreated group.

The effect of *Melia azedarach* on GPx, GST, SOD and catalase activity is shown in Table 2. It showed that GPx,

Table 1: Effect of *Melia azedarach* on some serum chemical parameters of paracetamol intoxicated rats.

Parameters	Normal control	Paracetamol	Extract + paracetamol	Extract	Silymarin
Bilurubin (mg/dl)	0.82 ± 0.21	1.98 ± 0.37°	1.45 ± 0.79**	1.01 ± 2.4**	1.02 ± 0.30**
SGOT (U/L)	59.12 ± 10.28	186.33 ± 16.77°	150.00 ± 2.66*	102 ± 3.20***	85.27 ± 10.53***
SGPT (U/L)	46.59 ± 6.71	99.13 ± 10.51°	79.04 ± 5.3*	63.69 ± 2.80***	51.08 ± 5.31***
ALP (U/L)	133.60 ± 1.50	373.94 ± 7.62°	225.00 ± 1.21**	181.00 ± 3.40***	153.52 ± 9.26***
Total protein (g/dl)	6.85 ± 0.13	4.98 ± 0.72°	6.69 ± 2.50*	6.75 ± 0.80*	6.72 ± 0.06**

Values are mean ± S.E.M. number of rats = 6. Paracetamol control group compared with normal, control group °$p < 0.001$. Experimental groups compared with Paracetamol control group *$p < 0.05$, **$p < 0.001$, ***$p < 0.001$.

Table 2: Effect of *Melia azedarach* extract on antioxidant enzymes in liver of rats.

Parameters	Normal control	Paracetamol	Extract + paracetamol	Extract	Silymarin
GPx (U^p/gp)	19.60 ± 1.20	11.00 ± 0.80*	18.2 ± 1.90	18.90 ± 0.70	20.20 ± 1.40
GST (U^q/mgp)	1290.00 ± 52.00	903.00 ± 52*	1187.00 ± 59.00	1342.00 ± 67.00	1302.00 ± 43.00
SOD (U^s/gp)	56.50 ± 2.30	39.60 ± 2.70*	52.20 ± 3.10	65.90 ± 1.80	62.70 ± 1.50
CAT (U^t/gp)	68.40 ± 3.90	52.10 ± 3.10*	66.70 ± 1.90	74.10 ± 2.30	67.20 ± 2.60

Values are mean ± SEM; $n = 6$ in each group. *Significantly different with compared to saline and other treatment groups. GPx = Glutathione peroxidase (U^p = µmoles GSH utilized/min). GST = Glutathione–S-transferase (U^q = nmoles CDNB-GSH conjugate/min). SOD = superoxide dismutase (U^s = ukat) CAT = Catalase (U^t = nmoles of H_2O_2 decomposed/sec).

1) Section of liver of control group

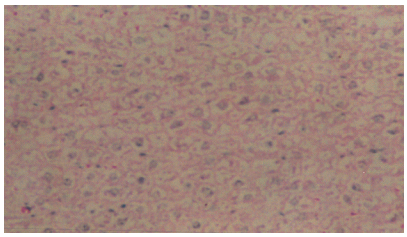


Figure 1: The parenchymatous tissue showing the regenerative changes by emptying of cytoplasm of hepatocytes and nucleus was centrally located.

2) Section of the liver of paracetamol treated group

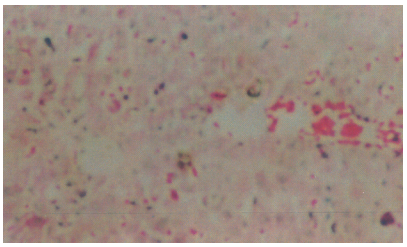


Figure 2: Liver parenchyma was flooded with the hemorrhage and the regression of hepatocytes was also seen

3) Section of liver of paracetamol and melia azedarach *treated group*

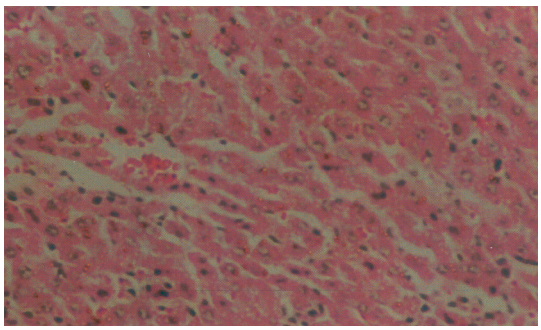


Figure 3: Liver showing mild congestion increased space of canaliculi moderate

4) Section of liver of melia azedarach *treated group*

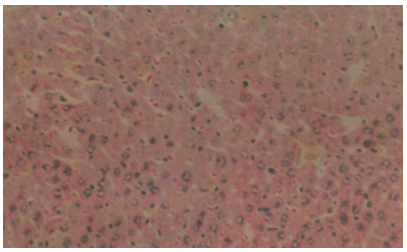


Figure 4: Hepatocytes were regenerative and a milder degree of vacuolation but prominent nuclei, indicating returning to normalcy

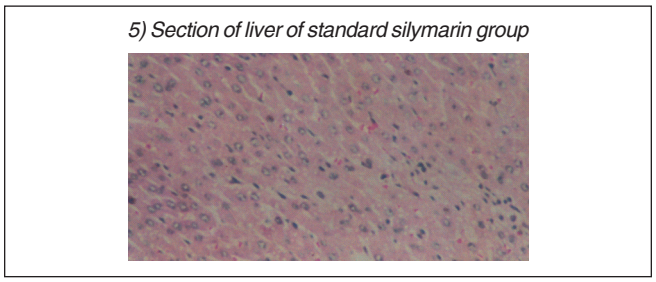


Figure 5: The sheets of hepatocytes were positioned in typical radiation pattern. Hepatocytes were individually demarked with canalicular space.

GST, SOD and catalase activity were significantly ($p < 0.001$) decrease in paracetamal-intoxicated rats when compared with those animals in normal control group. On the other hand, the group which received leaf extract and paracetamol (Group III), the values of above enzymatic parameters were near normal compared to Group I animals and were significantly different from their paracetamol treated control group (Group III Vs Group II). But there was no significant difference between Group I and Group IV animals. The results are well compared with Silymarin standard drug treated group (Group V).

DISCUSSION

Paracetamol is one of the most commonly used hepatotoxin. The covalent binding of N-acetyl-p-benzoquinoneimine (oxidation product of paracetamol) to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity induced by paracetamol have been reported earlier.[25,26]

Although serum enzyme levels are not a direct measure of hepatic injury they show the status of liver. The elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.[27] Thus lowering of enzyme content in serum is a definite indication of hepatoprotective action of a drug. High level of SGOT indicates liver damage such as due to viral hepatitis. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore SGPT is more specific to the liver and a better parameter for detecting liver damage.[28] Serum ALP and bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis in presence of increasing biliary pressure[29]

In the present study, *Melia azedarach* has been found to reduce SGOT, SGPT, ALP and bilirubin in the treated groups compared with the untreated one (Table 1). It is well documented that the hepatocellular enzymes (GPx, GST, SOD and CAT) serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity. Administration of *Melia azedarach* leaf extract significantly enhanced the hepatic level of glutathione dependent enzymes and superoxide dismutase and catalase activity (Table 2).

CONCLUSION

In conclusion, the results of present study demonstrate that *Melia azedarach* leaf extract has potent hepatoprotective activity against Paracetamol induced liver damage in rats. The results also imply that the hepatoprotective effects of *Melia azedarach* may be due to its antioxidant property. Further investigation is in progress to determine the exact phytoconstituent (s) responsible for hepataprotective effect.

ACKNOWLEDGMENTS

My sincere thanks to ***Dr. A Srinavasa Rao***, *Principal, Bhaskar Pharmacy College* and ***Dr. Mohammed Ibrahim***, *Principal, Nizam Institute of Pharmacy*, for rendering their suggestions and helping me in each and every step of completing this research work successfully.

REFERENCES

1. Simonsen L, Kan A, Lloy J. Unsafe infections in the developing world and transmission of bloodborne pathogens. *Bull World Health Organ* 1999; 77:789-800.
2. Gupta M, Mazumder UK, Sambath Kumar R, et al. Antioxidant and protective effects of Ervatamia coronaria Stapf., leaves against carbon tetrachloride – induced liver injury. *European Bull Drug Res* 2004a; 12:13-22.
3. Gupta M, Mazumder UK, Siva kumar T, et al. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride – induced liver damage in rats. *Iranian J Pharmacol Therapeutics* 2004b; 3:12-20.
4. Kukongviriyapan V, Janyacharoen T, Kukongviriyapan U, et al. Hepatoprotective and antioxidant activities of *Tetracera loureiri*. *Phytother Res* 2003; 17:717-721.
5. Sannigrahi S, Mazumder UK, Pal D, Mishra SL. Hepatoprotective potential of methanol extract of *Clerodendrum infortunatum* Linn. against CCl_4 induced hepatotoxicity in rats. *Phcog Mag* 2009;5:394-9.
6. Gutierrez RP, Navarro YG. Antioxidant and hepatoprotective effects of the methanol extract of the leaves of *Satureja macrostema*. *Phcog Mag* 2010; 6:125-31.
7. Bairwa NK, Sethiya NK, Mishra SH. Protective effect of stem bark of *Ceiba pentandra* linn. against paracetamol-induced hepatotoxicity in rats. *Phcog Res* 2010; 2:26-30.
8. Lata H, Ahuja GK. Role of free radicals in health and disease. *Ind J Physiol & Allied Sci*. 2003; 57:124.
9. Mazumder UK, Gupta M, Rajeshwar Y. Antihyperglycemic effect and antioxidant potential of *Phyllanthus niruri* (Euphorbiaceae) in streptozotocin induced diabetic rats, *European Bull Drug Res* 2005; 13:15-23.
10. Bhawna S, Kumar SU. Hepatoprotective activity of some indigenous plants. *Int. J. Pharm. Tech. Res* 2009;4:1330-1334.
11. Olaleye MT, Adegboye OO, Akindahunsi AA. *Alchornea cordifolia* extract protects wistar albino rats against acetaminopheninduced liver damage. *Afr. J. Biotechnol* (2006), 5(24):2439-2445.

12. H.P. Rang, M.M.Dale, J.M.Ritter, P.K.Moore *et al,* Pharmacology fifth edition. Page no: 251.
13. Meredith TJ, Vale JA, Proudfoot AT, Poisoning caused by analgesics. In: Oxford textbook of medicine. Weatherall DJ, Ledingham JGG Warrell DA, editors. 3rd ed. Oxford: 1996.
14. Wealth of India, raw material Vol-IV (L.M) Page no.323. [10a] Chemical constituents Page 324-325.
15. Corpinella MC, Miranda M, Almiron WR, Ferrayoli CG, Almedia FL, Palacios SM. (In vitro pediculicidal and ovicidal activity of an extract and oil from fruit of melia azedarach L). *J Am Acad Delmatol*, 2007; 56(2):250-6. Epub 2006 Decu. PMID: 17147968.
16. Wandscheer CB, Duque JE, Da Silva MA, Fukuyama Y. Wohlke JL, Adelmann J, Foutana JD. (Larvicidal action of ethanolic extracts from fruits endocarps of *melia azedarach* and *Azadirachta indica* against the dengue mosquito. *Aedes Aegypti. Toxicon*. 2004 Dec. 15; 44(8):829-35. PMID: 15530964.
17. Descalzo AM, cotoC. (Inhibition of the pseudorabies virus (scis herpesvinyl) by an andvital agent isolated from the leaves of *Melia azedarach. Rev. Argent microbial* 1989 Jul-Dec. 21(3-4):133-40, Spanish. PMID: 2562074.
18. Choudhary DN, Singh JN, Verma SK, Singh BP. (Antifertility effects of leaf extracts of some plants in male rats) *Indian J Exp*. Biol. 1990 Aug. 28(8)L 714-6.
19. Litchfield JT, Wilcoxon FA. Simplified method of evaluating dose effect exp eriments. *J Pharmacol Exp Ther* 1949; 96:99-133.
20. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin-phenol reagent. *J Biol Chem* 1951; 193:265-275.
21. Rotruck JT, Pope AL, Gouther HE, Swanson AB, Hateman Da, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase science 1973; 179:588-90.
22. Habig WH, Pabst MJ, Jokaby WB. Glutathione-s-transferase. The first enzymatic step in mercapturic acid formation. *J.Biol chem*. 1979; 249:7130-9.
23. Mishra HP, Fridovichl. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol chem*. 1972; 247:3170-5.
24. Tarkahara S, Hamilku BH, Nell JV, Kobra TY, Ogura Y, Nishimura ET. Hypocatalasemia. A new genetic carrier state. *J clin. invest*. 1960; 39 610-9.
25. Jollow DJ, et al. Acetaminophen induced hepatic necrosis: (II) role of covalent binding in vivo. *J pharmacol exp. ther* 1973; 187:195-9.
26. Wendel A, Feuerstein S, Konz KH. Acute paracetamol intoxication of starved. Mice leads to lipid peroxidation in vivo. *Biochem pharmacol* 1987: 28:2051-3.
27. Zimmerman HJ, Seeff LB. Enzymes in hepatic disease In: Goodly, E.E., (Ed), Diagnostic Enzymology, Lea and Febiger, *Philadelphia*. 1970, pp. 24-26.
28. Willianson EM, Okpako DT, Evans FJ. Selection, preparation and pharmacological evaluation of plant material, John Wiley, England, 1996, pp. 1.
29. Moss DW, Butterworth PJ. Enzymology and Medicine. Pitman Medical, London 1974, pp. 139.

In-Vitro antioxidant and free radical scavenging activity of *Butea Frondosa* Roxb. Flower.

Giridhari lal, Manasi Mantri*

Department of Pharmaceutical Chemistry, School of Pharmacy and Technology Management, SVKM's NMIMS University, Mumbai-400 056 India.

ABSTRACT

In this present study we aim to evaluate *in vitro* antioxidant and free radical scavenging potential of different extract of *Butea Frondosa Roxb.* Flowers in different system viz. Radical scavenging activity by DPPH reduction and lipid peroxidation assay (TBARS). All extracts have different level of antioxidant activity. The activity was expressed as the inhibitory concentration (IC_{50}). In conclusion the present study indicates that alcoholic extract and *n*-butanol fraction of alcoholic extract which is obtained by solvent partition of alcoholic extract of *Butea Frondosa Roxb.* flower showed significant activity by both methods and can be a potential source of natural antioxidants.

Key words: *Butea Frondosa Roxb.*, Antioxidant activity, DPPH radical, Lipid peroxidation assay and TBARS.

INTRODUCTION

In recent years, phyto-medicine is in great demand as food supplement for age related chronic diseases, because of their multi-targeted action and lesser side effects.[1] *Butea Frondosa Roxb.* (Family: Fabaceae) is commonly known as "palash". The Sanskrit word palasa literally means that which looks like a flesh or blood. It has various synonyms in Ayurvedic texts. Like kimsuka – the flowers resemble to the parrot's beak, triparna – 3 – foliate leaves, bija sneha – the seeds are oily and many other, describing its usefulness for yajna i.e. rituals and worships performed infront of fire viz. yajniya, samidvara, brahmapadapa, samiduttama etc. Maharsi Susruta has categorized it as visaghna (anti-toxin), sukrasodhana (purifies seminal fluids) and stambhana – astringent and anti-diarrhoeal. Two varieties of palasa – white flowered and red flowered, are mentioned in Samhita.[1]

It holds an important place because of its medicinal and other miscellaneous uses of economic value. Flowers are astringent to bowel, increase "Vata" cure "Kapha", leprosy, strangury, gout, skin diseases, thirst, burning sensation; flower juice is useful in eye diseases. Flower is bitter, aphrodisiac, expectorant, tonic, emmenagogue, and diuretic good in biliousness, inflammation and gonorrhoea. Flowers also show good antistress, anticonvulsive, antihepatotoxic, antiestrogenic and anthelmintic activity. The dye is useful in enlargement of spleen. Flowers are depurative, as a poultice they are used to disperse swelling and to promote menstrual flow.[1,2,3]

Phytochemical investigations of the dried flowers of *Butea frondosa Roxb* revealed the presence of at least seven flavones and flavanoid constituents and also four free amino acids. These includes Triterpene, butein, butrin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, monospermoside (butein 3-e-D-glucoside) and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin) and steroids.[4,5]

Oxygen involved in the respiratory process can be transformed under some condition into superoxide anion, hydroxyl radical, singlet oxygen and hydrogen peroxide.[6] These reactive oxygen species are implicated in some diseases such as inflammation, cancer, ageing, anaemia, degenerative diseases and atherosclerosis.[7] A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant property. Several studies have been to asses the antioxidant properties of natural products.[8] Scientific information on chemical constituent and antioxidant properties of various plants less widely used in the medicine is still rather scarce. Hence assessment of chemical constituent and such properties remain an interesting and useful task particularly for finding new source

***Address for correspondence:**
E-mail: mantrimanasi@yahoo.co.in

DOI: 10.5530/pj.2011.21.11

of natural antioxidant, functional food and neutraceuticals including polyphenolic such as flavonoid, tannin, proanthocyanidin.[9] The objective of the present study was to investigate the *in vitro* antioxidant activity of alcoholic extract of *Butea Frondosa Roxb.* including its *n*-butanol and water fraction.

MATERIALS AND METHODS

Chemicals

1, 1-diphenyl-2-picryl hydroxyl (DPPH) purchased from Sigma Aldrich Ltd. Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were purchased from Loba chemie laboratory reagents. Potassium chloride purchased from Merck Ind. Ltd. All other chemicals or solvents either used for extraction or assay purpose were purchased from Qualigens fine chemicals. All the chemicals required are of analytical grade.

Plant material and extraction

Butea Frondosa Roxb. Flowers were purchased from D.G. Ayurvedic Sangrah Andheri, Mumbai, India and the voucher specimen (No. 268) was deposited in the department for future reference.

The flowers were further dried in shadow and powered. The powder obtained was passed through sieve no. 85, weighed and stored in tightly closed container. 250 gm of coarsely powdered flowers of *Butea frondosa* were subjected to simple percolation method of extraction with 3 different solvent viz. 95% ethanol, methanol and water separately for 72 hours. The resulting extracts were concentrated under reduced pressure using rotary vacuum evaporator at 40-50°C to get the semisolid mass. These masses were transferred in petridish & allowed to dry in an oven for about 2 to 3 hours. Maximum yield as well as no. of compounds was obtained in ethanolic extract. Hence ethanolic extract selected for evaluation of antioxidant activity.

For initial screening investigations 10 gm of the total ethanolic extract was partitioned between water and ethyl acetate (3 times). After removal of the ethyl acetate fraction, the remaining water phase was treated with *n*-butanol (3 times). Both the ethyl acetate and the *n*-butanol fractions were dried with sodium sulphate. The solvent was removed by drying in oven at 40-50°c to get the semisolid masses of ethyl acetate, water and *n*-butanol fraction. Each fractions obtained were tested for presence of various phytoconstituents with the help of TLC. Finally whole ethanolic extract, and *n*-butanol fraction & water fraction of ethanolic extract were selected for evaluation of antioxidant activity. Percentage yield of the alcoholic extracts was found to be 16.37%.

Free radical scavenging activity

DPPH scavenging activity

The free radical scavenging activity was measured *in vitro* by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Blois.[10] About 0.36 mg/ml solution of DPPH in 100% methanol was prepared and 200 µl of this solution was added to 2.7 ml of the extract dissolved in methanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 20 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC_{50} value of the crude extract was compared with that of ascorbic acid, which was used as the standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radicals was calculated using the following formula,

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A Test}}{\text{A control}} \times 100$$

Where,

A control is the absorbance of the control reaction mixture.
A test is the absorbance of sample of the extracts at different concentrations.

Graph of % Inhibition vs. Concentration was plotted to obtain IC_{50} value.

Lipid peroxidation inhibitory activity by TBARS method

Antilipid peroxidation abilities of *Butea frondosa* was evaluated by Thiobarbituric acid reacting substances (TBARS) using the method of Ohkawa.[11] It is one of the most frequently used tests for measuring the peroxidation of lipids. This method is carried out on liver homogenates of wistar strain albino mice. Curcumin was used as reference standard.

Mouse was sacrificed using anaesthetic ether. The liver was quickly removed and chilled in ice cold saline. After washing with ice cold saline the liver was homogenized in 0.15 M KCl to get 10% liver homogenate.

Fresh liver homogenate (0.2 ml) was mixed with 0.15 M KCl (0.1 ml) and Tris buffer (0.4 ml). The extracts were then added in various concentrations. *In vitro* lipid peroxidation was initiated by addition of ferrous sulphate (10 mM) and ascorbic acid (100 mM), 0.1 ml each. After incubation for 1 hour at 37°C, reaction was terminated by addition of Thiobarbituric acid reagent (2 ml) and boiled for 15 minutes for development of coloured complex. Tubes were centrifuged at 4000 rpm for 10 minutes and cooled. The colour was estimated spectrophotometrically at 532 nm. % reduction of thiobarbituric acid reacting substances was calculated with respect to control to which no sample has been added. The inhibition of lipid peroxidation was

determined by calculating the % reduction in formation of TBARS and expressed in terms of IC_{50} (Concentration in mg required for a 50% decrease in absorbance of malondialdehyde).

$$\% \text{ Inhibition} = \frac{\text{A control} - \text{A Test}}{\text{A control}} \times 100$$

Where,

A control is the absorbance of the control reaction mixture.
A test is the absorbance of sample of the extracts at different concentrations.

Graph of % Inhibition vs. Concentration was plotted to obtain IC_{50} value.

Table 1: The IC_{50} value of various extract by DPPH Method

Fraction	IC_{50} Value
Ascorbic acid	14 µg/ml
Whole alcoholic extract	130 µg/ml
n-butanol fraction of alcoholic extract	150 µg/ml
water fraction of alcoholic extract	198 µg/ml

Table 2: The IC_{50} value of various extract by TBARS Method

Fractions	IC_{50} Value
Curcumin	10 µg/ml
Whole extract	55 µg/ml
n-butanol fraction	137 µg/ml
Water fraction	227 µg/ml

RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed that flowers of *B. Frondosa* are well known source of flavones and flavonoid compounds and these compounds have been reported to possess antioxidant activity. So the extracts were evaluated for *in vitro* antioxidant activity.

Reactive oxygen species (ROS) contribute to a great variety of diseases. ROS including hydrogen peroxide, superoxide radical anion, nitric oxide, singlet oxygen react with biological molecules leading to cell and tissue injury.[12] Plants exhibit efficient antioxidant activity owing to their phenolic constituent.[13] Antioxidant activity of each extract was investigated by using different models.

DPPH radical scavenging activity[2,19]

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. It is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to overall antioxidant capacity of the sample.

The odd electron in DPPH free radical shows a strong absorption band at 517 nm (in methanol or ethanol) and its solution appears as deep violet colour. As this electron becomes paired with hydrogen from a free radical scavenging antioxidant, the absorption vanishes i.e. colour changes from violet to yellow. The molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640. The resulting decolourization is stoichiometric with respect to the number of electrons captured.

The IC_{50} values of Ascorbic acid, alcoholic extract and *n*-butanol & water fraction of alcoholic extract found to be 14, 130, 150, 198 µg/ml respectively. The whole alcoholic extract has lower IC_{50} value among the extracts and hence maximum antioxidant activity.

Lipid peroxidation inhibitory activity by TBARS method

This method is based on the formation of malondialdehydes during lipid peroxidation. Method involves isolation of microsomes from rat liver and induction of lipid peroxides with ferric ions leading to the production of small amount malondialdehyde (MDA). Peroxidation was induced in liver tissue by iron sulphate in presence of ascorbic acid. Thus free radicals are generated by ferrous – ascorbate system. Malondialdehyde (MDA) Forms a 1:2 adduct with thiobarbituric acid and produces pink coloured complex, which can be measured at 532 nm spectrophotometrically.

The results demonstrated an anti-oxidative effect of the herbal compound as indicated by reduced MDA levels. Ex-vivo T-BARS Method confirms the antioxidant activity of "Flower part of *Butea Frondosa*" and proves to have potent antioxidant activity.

CONCLUSION

In case of the extracts of *B. Frondosa*, the most potent antioxidant activity was observed in whole extract followed by *n*-butanol fraction by DPPH method as the minimum concentration of 130 µg/ml of whole extract and 150 µg/ml of *n*-butanol fraction required for the 50% reduction of absorbance. The activity was also evaluated by TBARS method, minimum concentration of 55 µg/ml of whole extract and 137 µg/ml of *n*-butanol fraction required for the 50% reduction of absorbance. The significant antioxidant activity of *n*-butanol fraction may be due to the extraction of more flavanoid in this fraction.

REFERENCES

1. Burlia DA, Khadeb AB, A Comprehensive review on *Butea monosperma* (Lam.) Kuntze. Pharmacognosy Reviews. Jul-Dec 2007, 1(2):340-345.
2. Chu YH, Chang CL, Hus HF, Flavanoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric. 2000;80:561-566.
3. Cimanga K, Ying L, Bruyna DE, Hermans N, Bakana P, Tona L, Kamba DT, Kalenda H, Pietrs L. Radical scavenging & Xanthine oxides inhibitory activity of phenolic compounds from *Bridelia ferruginea* steam bark. J. Pharmacy and Pharmacol. 2001;53:757-761.
4. Corona-Bustamante A, Viveros-Paredes JM, Flores-Parra A, Lilia A, Antioxidant Activity of Butyl- and Phenylstannoxanes Derived from 2-, 3- and 4-Pyridinecarboxylic Acids. Molecules. 2010; 15:5445-5459.
5. Dissanayake DP, Abeytunga DTU, Vasudewa NS, Ratnasooriya WS, Inhibition of lipid peroxidation by extracts of *Pleurotus ostreatus.* Pharmcognosy Magazine. 2009; 5(19):266-271.
6. Gilbanananda R, Hussain SA, Oxidant, antioxidant & carcinogenesis. Ind. J. Exp. Biol. 2002; 40:1213-1232.
7. Kumar D, Mishra SK, Tandan SK, Tripathi HC. Possible Mechanism of Anthelmintic Action of Palasonin on Ascaridia Galli. Indian Journal of Pharmacology. 1995; 27:161-166.
8. Wagner H, Geyer B, Fiebig M, Kiso Y, Hikino H. Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers. Planta Medica. 1986; 2:77-79.
9. Monika Verma et al, Chemistry and biology of the Indian dhak tree *Butea monosperma.* Journal of Medicinal and Aromatic Plant Sciences. 1998; 20:85-92.
10. Miliauskas G, Venskulonis PR, Beak TA, Screening of radical scavenging activity of some medicinal and aromatic plants extract. Food Chemistry. 2004; 85:237-437.
11. Janet Calero Sánchez, Roberto Faure García, Ma. Teresa Mitjavila Cors, 1,1-Diphenyl-2-picrylhydrazyl radical and superoxide anion scavenging activity of *Rhizophora mangle* (L.) bark. Phcog. Res. 2010; 2(5):279-284.
12. Tenpe CR, Upaganlawar A, Bhagat A, Yeole PG, *In-Vitro* antioxidant and free radical scavenging activity of *Jasminum sambac Linn.* Leaves. Phcog Mag. 2008; 4(15):124-129.
13. Ohkawa H, Onishi N, Yagi K, Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351-358.
14. Groot H, Raven J, Tissue injury by reactive oxygen species & the protective effect of flavonoids. Fund. Clin. Pharmacol. 1998; 12:249-255.
15. Milovanovic KP, Jovanovic V, Djermanovic M, Antioxidant activity of the constituent of Picris echoides. J. Serb. Chem. Soc. 2002; 66(10):7-15.
16. Pandey N, Tripathi YB, Antioxidant activity of tuberosin isolated from *Pueraria tuberose Linn*. Journal of Inflammation. 2010; 7(47):1-8.
17. Yuan C, Chan YP, Chang J, Antioxidant activity of extract from *Polygonum cuspidatum*, BiolRes. 2007; 40:13-21.
18. Ullah O, Sultana S, Haque A, Tasmin S, Antimicrobial, Cytotoxic and Antioxidant Activity of *Centella asiatica. E*uropean Journal of Scientific Research.2009; 30(2):260-264.

Anti-Staphylococcus Activity of Uruguayan Riverside Forest Plants.

Stephanie Barneche[a]; María P.Cerdeiras[b]; Rodrigo Lucarini[c]; Carlos H.G. Martins[c]; Cristina Olivaro[a], Alvaro Vazquez[a*]

[a]*Catedra de Farmacognosia, Facultad de Quimica, UDELAR. CC 1157,Montevideo, Uruguay.*
[b]*Catedra de Microbiología, Facultad de Quimica, UDELAR. CC 1157, Montevideo, Uruguay.*
[c]*Universidade de Franca, Laboratório de Pesquisa em Microbiologia Aplicada, LaPeMA, Franca-SP, Brasil.*

ABSTRACT

Methicillin resistant *Staphylococcus aureus* is a major nosocomial pathogen which causes increased morbidity and mortality worldwide. Interest in plants with antimicrobial properties has thus revived as a consequence of current problems associated with the use of antibiotics. In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin. Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization. Extracts that were positive in the qualitative diffusion tests against *S. aureus* were selected for a more detailed study. The objective of the present work is to study the anti MRSA activity of 37 plant extracts that have previously shown activity against *S. aureus* in a qualitative diffusion assay. The plant material were separately twice extracted by maceration with EtOH/H_2O 70:30, acetone and $CHCl_3$. Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and Laboratory Standards Institute using sensitive (ATCC 6538p) and resistant (ATCC 43300, ATCC 700699 and USA 100) strains. Some of the extracts showed good activity specially species of the Myrtaceae family with MICs as low s 7.8 μg/mL. These promissory results were obtained encourage further work to be done regarding the isolation and structural elucidation of the active compound(s).

Key words: Antibacterial activity Methicillin resistant *S. aureus*, Myrtaceae

INTRODUCTION

In spite of the great advances in chemotherapeutics, infectious diseases are still one of the leading causes of death in the world. The World Health Organization[1] states that infectious and parasitic diseases account for nearly 11 million among the 57 million total deaths in 2003. Although there seems to be a great array of antibacterial and antifungal drugs in clinical use, the appearance of resistant organisms makes them sometimes ineffective or lead to recurrence.[2,3] Amongst some of the most problematic clinically relevant pathogens at present, methicillin-resistant *Staphylococcus aureus* (MRSA) ranks as one of the most difficult bacteria to treat.[4, 5]

The use of higher plants and preparations made from them to treat infections is an age-old practice in a large part of the world population, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases.[6-8] Interest in plants with antimicrobial properties has revived as a consequence of current problems associated with the use of antibiotics.[9-15]

Although Uruguay is usually considered a grassland country it has more than 30 woody families and a considerable amount of native forests, especially along river banks [gallery forests) and "quebradas" (gulch forests), with a subtropical and tropical vegetation intrusion. These forests comprise more than 810.000 has with a varied and distinctive flora, around 250 comprising woody and arbustive species.[16] The riverside forest along the Uruguay River banks is especially interesting as a great number of tropical species original from the Chaco and Espinal floristic provinces are also present.[17]

In previous work we undertook the biological and chemical prospection of the gallery forest of the northern Uruguay River basin.[18] Plants were selected after an exhaustive review of the available literature according at its ethnopharmacological use and submitted to antimicrobial assays and phytochemical characterization.[19] Extracts that were positive in the

***Address for correspondence:**
Phone +598 2 9244068; Fax +598 2 9241906
E-mail: avazquez@fq.edu.uy

DOI: 10.5530/pj.2011.21.12

qualitative diffusion tests against *S. aureus* were selected for a more detailed study.

In this work, we present the results of the antimicrobial activity of these extracts against methicillin sensitive and resistant *S. aureus* strains.

MATERIAL AND METHODS

Plant Material

Plants were collected during the 2006, 2007 and 2008 spring-summer season in different locations along the Uruguay River shore between the Chapicuy stream confluence and river Guaviyu confluence, near Paysandù and along the banks of Queguay river between the confluence of Queguay Chico y Queguay Grande and it`s opening in the Uruguay river. Plants were identified by Lic. F. Haretche, Museo y Jardin Botanico "Atilio Lombardo", Montevideo. Voucher specimens are kept in the MVJB Herbarium, Jardin Botanico, Montevideo.

Extraction

The plant material was air dried in the dark and milled to a coarse powder. Samples (20 g) were separately twice extracted by maceration with EtOH/H_2O 70:30, acetone and $CHCl_3$ (100 mL) for 48 h. Combined extracts were evaporated under vacuum and lyophilised when necessary.

Microbiological Assay

Minimum inhibitory concentration (MIC) was determined by the microdilution technique according to Clinical and

Table 1: Antimicrobial activity of plant extracts

			Strains[c]/MIC (µg/mL)			
Plant Species	Used part[a]	Extract[b]	ATCC 6538p	ATCC 700699	ATCC 43300	USA 100
Allophylus edulis	L	3	>500	>500	>500	>500
Combretum fruticosum		2	500	250	500	250
Croton tenuissimus		2	>500	500	500	250
Dolichandra cynanchoides		1	>500	>500	>500	>500
Eugenia mansoni		1	250	250	250	>500
		2	7.8	7.8	7.8	7.8
		3	7.8	7.8	7.8	7.8
Eugenia uruguayensis		2	31.3	31.3	31.3	31.3
		3	31.3	31.3	31.3	15.6
Gleditsia amorphoides m		1	>500	>500	>500	500
Gouania ulmifolia		2	>500	>500	>500	>500
		3	>500	>500	>500	>500
Guettarda uruguensis		2	>500	>500	>500	>500
Hexachlamys edulis	L	2	500	500	500	>500
Maytenus illicifolia	L	2	>500	>500	>500	250
Mimosa uraguensis	L	1	>500	>500	>500	125
		2	>500	>500	>500	>500
Myrcianthes cisplatensis	L	1	62.5	31.3	31.3	>500
		2	7.8	7.8	15.6	6.25
		3	125	250	62.5	2.5
	F	1	250	250	250	62.5
		2	125	62.5	125	31.3
		3	62.5	31.3	62.5	250
Myrrhinium atropurpureum	L	1	125	125	250	15.6
		2	125	125	250	62.5
Myrsine venosa	L	1	>500	>500	>500	>500
Ocotea acutifolia	L	2	>500	>500	>500	>500
		3	>500	>500	>500	>500
Phyllanthus sellowians	L	2	>500	250	250	>500
Polygonum punctatum	AP	3	>500	500	500	>500
Pouteria salicifolia	L	2	>500	>500	>500	>500
Psychotria carthagenensis	L	1	500	>500	>500	250
Ruprechtia laxiflora	L	2	>500	>500	500	>500
Scutia buxifolia		1	>500	>500	500	250
		2	>500	>500	>500	>500
Terminalia australis	L	2	>500	250	MIC	500
Urvillea uniloba	AP	2	>500	>500	>500	>500

[a]Used Part: L leaf, F: fruit, AP: aerial parts
[b]Extraction solvent: 1 EtOH-H_2O 70:30, 2 CH_3COCH_3, 3 CH_2Cl_2
[c]Strains : ATCC 6538p (*Staphylococcus aureus* ATCC 6538p MSSA), ATCC 700699 (*Staphylococcus aureus* ATCC 700699 MRSA)
ATCC 43300 (*Staphylococcus aureus* ATCC 43300 MRSA), USA 100 (*Staphylococcus aureus* USA 100 New York-Tokio clone).

Laboratory Standards Institute [20] using sensitive (ATCC 6538p) and resistant (ATCC 43300, ATCC 700699 and USA 100) strains.

RESULTS AND CONCLUSION

The results of the antibacterial assays are depicted in Table 1. As can be seen 19 out of 37 (50%) of the extracts showed measurable activity with MICs below 500 μg/mL. This is an expected result as the samples were carefully selected in an ethnobotanical survey and went through a pre-screening process [18, 19].

In particular extracts of *Eugenia mansoni*, *Eugenia uruguayensis, Myrcianthes cisplatensis* and *Myrrhinium atropurpureum*, all belonging to the Myrtaceae Family, showed MICs as low as 7.8 μg/mL with several of the resistant strains.

The MRSA chosen were not only resistant to methyciline, but also to oxacilline (ATCC 43300), to vancomycine (ATCC 700699) and multiresistant (spectinomycine, clindamycine, erythtromycine, vancomycine reduced susceptibility) the USA 100.[21-23]

Results obtained for *Myrcianthes cisplatensis* fruit extracts EtOH-H_2O and CH_3COCH_3 are very interesting as they show higher activity for the multiresistant *S. aureus* USA 100 than against all other *S. aureus* strains assayed. It had shown a broad spectrum of antimicrobial activity for all its extracts, even including fungi and *Mycobacterium*, in this case with MIC values of 100-200 μg/mL. Results suggest that the compounds responsible for these antimicrobial activities most probably belong to at least two different groups with different solvent solubilities, as the extracts with antimycobacterial activity are those obtained with chlorophorm and acetone, whilst the most polar extract showed activity against *S. aureus* USA 100.

ACKNOWLEDGEMENTS

This work was supported by the State of São Paulo Research Foundation (FAPESP) – 2009/18278-0 and 2010/02066-0 and University of Franca (Br) and PEDECIBA program and the national Agency for Research (ANII) (Uy). The authors gratefully acknowledge Prof. Agnes S. Figueiredo for technical advice.

REFERENCES

1. World Health Organization. The world health report 2007 - A safer future: global public health security in the 21st century. Geneva (Switzerland): WHO; 2007.
2. World Health Organization. Antimicrobial Resistance. Fact Sheet 194. 2002. [Cited on 20 May 2010]. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/.
3. Leggiadro RJ. Emerging drug-resistant bacteria: the wake-up call has come Southern Medical Journal1995; 88:884-6.
4. Michel M, Gutmann L. Methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci: Therapeutic realities and possibilities. Lancet 2002; 349:1901-1906.
5. Clarck AM. Natural products as a resource for new drugs. Pharm Res 1996; **13**:61-70.
6. Cox PA. Ethnopharmacology and the search for new drugs. In: Chadwick DJ, Marsh J, Editors. Bioactive compounds from plants. Chichester (UK): John Wiley & Sons:, p. 40-48; 1990.
7. Cassady JM, Baird WM, Chang CJ. Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. J Nat Prod 1999; 53:23-41.
8. Lewis WH, Elvin-LewisMP . Medicinal plants as sources of new therapeutics. Ann Mo Bot Gard 1995; **82**:16-24.
9. Cherigo L, Pereda-Miranda R, Gibbons S. Bacterial resistance modifying tetrasaccharide agents from *Ipomoea murucoides.* Phytochem 2009; 70:222-227.
10. Potterat, O, Hamburger M. Drug discovery and development with plant-derived compounds. Prog Drug Res 2008; 65:47-118.
11. Chaudhary S, Negi A, Dahiya V. The study of in vitro antimicrobial activity and phytochemical analysis of some medicinal plants in Chamoli Garhwal Region. Phcog J 2010; 2:481-485.
12. Sousa EA, Silva NF, Rodrigues F, Campos A, Lima S, Costa JG. Chemical composition and resistance-modifying effect of the essential oil of *Lantana camara.* Phcog Mag 2010; 22:79-82.
13. Al-Backri AG, Othman G, Afifi FA. Determination of the antibiofilm, antiadhesive, and anti-MRSA activities of seven *Salvia* species. Phcog Mag 2010; 24:264-270.
14. Adu F, Gbedema SY, Annan K. Antimicrobial and Resistance Modulatory Activities of *Corynanthe pachyceras.* Phcog Res 2009; 1:280-284.
15. Vieira A. A comparison of traditional anti-inflammation and anti-infection medicinal plants with current evidence from biomedical research: Results from a regional study. Phcog Res 2010; 2:293-295.
16. Escudero R. Proyecto Combinado GEF/IBRD Manejo Integrado de Ecosistemas y Recursos Naturales en Uruguay Componente "Manejo y Conservación de la Diversidad Biológica" Subcomponente Bosque nativo. Montevideo: MGAP; 2004.
17. Grela IA. Geografía florística de las especies arbóreas de Uruguay: Propuesta para la delimitación de dendrofloras. Tesis de Maestría en Ciencias Biológicas. Montevideo: MEC-UdelaR; 2004.
18. Bertucci A, Olivaro C, Almeida da Silva P, Ramos D, Cerdeiras MP, Vázquez A. Initial antimicrobial activity studies of plants of the Riverside forests of the southern Uruguay River. Rev Bras Farmacog 2009; 19:20-25.
19. Bertucci A, Olivaro C, Haretche F, Vazquez A. Prospección química del bosque de galería del río Uruguay. Rev Bras Farmacog 2008; 18:21-25.
20. Clinical and Laboratory Standards Institute ***M7-A7***—Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Wayne: CLSI; 2006.
21. Aires de Sousa M, de Lencastre H, Santos Sanches L, Kikuchi K, Totsuka K, Tomasz A. Similarity of antibiotic resistance patterns and molecular typing properties of methicillin-resistant *Staphylococcus aureus* isolates widely spread in hospitals in New York City and in a hospital in Tokyo, Japan. Microb Drug Resist 2002; 6:253-258.
22. Hiramatsu K, Hanaki K, Ino T, Yabuta T, Oguri K, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 2002; 40:135-136.
23. Thornsberry C, Mc Dougal L. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) *staphylococci.* J Clin Microbiol 1983; 18:1684-1691.

Antibacterial screening of the stem bark and leaf extracts of *Litsea glutinosa (Lour.)* C.B. Rob – an ethnomedicinally important tree of the Western Ghats

K. Pradeepa[1], V. Krishna[*], Venkatesh[1], K. Girish Kumar[1], B.V.Thirumalesh[2] and K.J. Naveen Kumar[2]

[1]P.G. Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnana sahyadri, Shankaraghatta – 577 451, Karnataka, India. [2]P.G. Department of Studies and Research in Microbiology, Kuvempu University, Jnana sahyadri, Shankaraghatta – 577 451, Karnataka, India.

ABSTRACT

Introduction: *Litsea glutinosa (Lour.)* C.B.Rob., is an aromatic medicinal tree belongs to the family Lauraceae and rarely distributed in the Western Ghats. Ethnomedicinally, the bark is used by the traditional practitioners as a demulcent, emollient and in the treatment of diarrhea and dysentery, and to facilitate wound healing process. The leaf paste is applied to relieve respiratory diseases; cough and also used as a demulcent and an emollient. **Methods:** The petroleum ether, chloroform and ethanol extracts of stem bark and leaf were screened for potential antibacterial activity using agar well diffusion method against 8 clinical strains isolated from infectious sources belonging to Gram-positive *Staphylococcus aureus, Bacillus stubtilis* and gram-negative *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi* and *Proteus sp*. Minimum Inhibitory Concentration (MIC) of the three sequential extracts of the leaf and stem bark was carried out against clinical isolated strains using two fold agar dilution method. **Results:** Ethanol extract of stem bark exhibited significant antibacterial activity with 2.5mg/ml MIC against *Bacillus subtilis* (19.20 ± 1.52), *Escherichia coli* (16.40 ± 0.55) and *Staphylococcus aureus* (15.20 ± 0.84) indicating the potent drug for the treatment of diarrhea and dysentery. Among the tree sequential extracts of the leaf the ethanol extract showed potent antibacterial activity against *Klebsiella pneumoniae* (16.40 ± 0.80) and may acting as a suitable drug for respiratory disorders. **Conclusion:** The obtained results justify the ethnomedicinal claims of *Litsea glutinosa*.

Key words: Agar-well diffusion, Clinical isolates, Lauraceae, Minimum inhibitory concentration, Two fold agar dilution.

INTRODUCTION

In recent years there has been a rising interest in the discovery of new antimicrobial compounds, due to alarming increase in the rate of infections with multi-drug resistant microorganisms.[1] The increased prevalence of antibiotic resistance bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control bacterial diseases.[2] In the present scenario due to emergence of multiple drug resistance to human pathogenic bacteria and fungi, especially the antibiotic penicillins, cephalosporins and chloromphenical types involve the enzymic inactivation of the antibiotic by hydrolysis or by the formation of an active derivative.[3]

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day.[4] In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world.[5] The pathogenic microbes like *Escherichia, Klebsiella, Proteus, Staphylococcus, Bacillus, Salmonella* and *Pseudomonas* species are implicated to cause several infections in human, as they are found in multiple environmental habitats.[6,7] Natural products especially, those used in ethnomedicine provide a major source of innovative therapeutic agents for various conditions including infectious diseases.[8] Recently, herbal medicines have increasingly been used to treat many difficult diseases including several infections. Ethnobotanical reports suggested that the herbal extracts and their constituents have been increasingly used to treat many infectious diseases[9,10] and

***Address for correspondence:**
E-mail: krishnabiotech2003@gmail.com

DOI: 10.5530/pj.2011.21.13

plants are the sleeping giants of pharmaceutical industry[11] and provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally.

Litsea glutinosa is an aromatic evergreen medium-sized tree belongs to the family Lauraceae and found to be very rare medicinal plant in the Western Ghats. The traditional practitioners residing in the vicinity of forests of the Bhadra Wild Life Sanctuary of the Western Ghats are using the stem bark and leaf extracts as a demulcent and mild astringent for diarrhea and dysentery,[12] the bark paste is applied to facilitate wound healing process.[13] Leaf juice used to relieve respiratory disorders, roots are used for poulticing sprains and bruises, and in wounds[12] and the berries yield oil which is used by some tribal practitioners in the treatment of rheumatism. The leaf juice for the treatment of the spontaneous and excessive flow of semen in young boys.[14]

Phytochemical screening of the stem bark revealed the presence of tannin, β-sitosterol, and actinodaphnine Boldine, norboldine, laurotetanine, n-methyllaurotetanine, *n*-methylactinodaphnine, quercetin, sebiferine, litseferine etc,[15] and two aporphine alkaloids, namely litseglutine A and B.[16]

So in the view of the above, the determination of potential antibacterial activity of *L. glutinosa* extracts could be more informative for the future use in clinical treatment as natural antimicrobial agent. In the present study the antibacterial activities of the sequential extracts of the leaf and stem bark was screened against 8 pathogenic clinical isolates of bacteria.

MATERIALS AND METHODS

Plant material and extraction

The leaves and stem bark of *L. glutinosa* were collected in October, 2010 from the Bhadra wild life sanctuary of the Western Ghats, Karnataka, India, and identified by *Tariq Husain*, Head and Scientist, Biodiversity and Angiosperm Taxonomy, National Botanical Research Institute, Lucknow, India and the voucher specimen (No. 97294) is deposited. Plant materials were cleaned with deionized water and dried at shade for a week, grounded mechanically and the powder was stored in air tight container. One kg of the powdered materials of each were refluxed in Soxhlet apparatus for 48 hrs in batches of 250 g each and sequentially extracted with the solvent petroleum ether, chloroform and ethanol. The solvent was evaporated and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland), allowing the solvent to completely evaporate on a water bath then finally vacuum dried. 400 mg of crude extracts of petroleum ether, chloroform and methanol were reconstituted with 10% Dimethyl sulphoxide (DMSO). The standard antibacterial drug ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka) was also tested at 1 mg/ml concentration.

Test microorganisms

The bacterial strains used for screening antimicrobial activity were collected from different infectious status of patients who had not taken any antibacterial drugs for at least two weeks with the help of an authorized physician, in the district health center of Shivamogga, Karnataka State, India (Table 1). The clinical isolates were identified following a standard method (Cowan *et al.* 1993.[17] The bacterial suspensions were diluted in 10^{-1} to 10^{-8} phosphate buffered saline. Samples were homogenized and then loaded in six aliquots of 20 µl each onto nutrient agar plates (agar, 15 g/L, beef extract 1 g/L, peptone 5 g/L, NaCl 5 g/L, yeast extract 2 g/L; diameter 55 mm, final pH 7.0 ± 0.2).

The 8 clinically isolated bacterial cultures of both gram-postive and gram-negative bacterial strains used for screening are: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi* and *Proteus sp.* The working cultures were prepared by inoculating a loopful of each test microorganism in 3 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 24 hours. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU/ml.

Determination of minimum inhibitory concentrations (MICs)

The agar dilution susceptibility test was performed based on modified method of NCCLS and CLSI[18,19] to determine the MIC. The Extracts dissolved in sterilized DMSO (400 mg/ml concentration) were taken as standard stock. A series of two fold dilutions of each extract in the final concentration of 40, 20, 10, 5 and 2.5 mg/ml were prepared in nutrient agar. After solidification, the plates were spotted with 100 µl of overnight grown bacterial cultures

Table 1: Profile of the clinical strains used for antibacterial activity

Clinical strains	Clinical condition	Source
Staphylococcus aureus	Food poisoning	Stool
Klebsiella pneumoniae	Pneumonia	Sputum
Pseudomonas aeruginosa	Burns	Mucus
Salmonella typhi	Food poisoning	Blood
Salmonella paratyphi	Hydronephrosis	Urine
Proteus sp.	UTI	Urine
Escherichia coli	Foos poisioning	Stool
Bacillus subtilis	Foos poisioning	Stool

approximately containing 1 × 10^4 CFU/ml. The test was carried out in triplicates. The plates were incubated overnight at 37°C. After 18 – 24 hours, the MIC was determined.

Screening for antibacterial activity

A sensitive radial diffusion technique was used for the assessment of antibacterial activity of the test samples. Sterilized nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 200 µl of test strain (1 × 10^6 Cfu/ml) of 24 h-incubated cultures of the clinical isolates were spread separately on the agar medium. Wells were created using a stainless steel sterilized cork borer (6.0 mm) under aseptic conditions. 100 µl of the plant extract at a concentration of 80 mg/ml were loaded into wells.10% DMSO was used as negative control and the fluoroquinolone antibiotic Ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka)) was used as the standard (100 µg/100 µl in sterilized distilled water) concomitantly with the test samples. The plates were incubated for 24 h at 37°C and the diameter of the zone of complete inhibition of the bacteria was measured around each well and readings were recorded in mm.

Statistical analysis

The experiments were carried out in triplicates and the mean values of zone of inhibition were evaluated after 24 hrs of incubation. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard deviation of zone of inhibition values between the extracts against bacterial culture.

RESULTS AND DISCUSSION

The Soxhlet extraction of 1000 g of leaf powder yielded 2.72% w/v of petroleum ether, 1.4% w/v g of chloroform and 13.62% w/v of ethanol extract respectively. And the yield of stem bark extracts were 0.46% w/v of petroleum ether, 0.76% w/v g of chloroform and 23% w/v of ethanol extract respectively.

The MIC values of 6 extracts ranged from 2.5 to 20 mg/ml [Table 2]. Petroleum ether extract of leaf showed maximum activity with MIC 10 mg/ml for *Salmonella typhi,* and petroleum ether extract of stem bark extract showed 5 mg/ml MIC for *Bacillus subtilis*. Chloroform extract of leaf showed 5 mg/ml MIC for *Bacillus subtilis,* and chloroform extract of stem bark extract showed 2.5 mg/ml MIC for *Bacillus subtilis* and *Klebseilla pneumoniae*. And ethanol extract of ethanol exhibited 2.5 mg/ml MIC for *Klebseilla pneumoniae.* In general, all extracts showed less than 40 mg/ml MIC for the tested bacterial pathogens. Interestingly, ethanol extract of stem bark extract had higher activity in *Bacillus subtilis, Escherichia coli, Staphylococcos aureus, Klebseilla pneumoniae and Salmonella typhi* even at a low concentration of 2.5 mg/ml.

The antibacterial activity of the organic solvent extracts showed varying magnitudes of inhibition patterns with standard positive control depending on the susceptibility of the tested microorganism. The mean inhibitory zone of six solvent extracts against 8 bacterial species is summarized in Table 3.

The analysis of petroleum ether extract of leaf against clinical bacterial pathogens showed a significant level of inhibition against *Salmonella typi* (5.75 ± 0.50), and petroleum ether extract of stem bark showed high activity against *Bacillus subtilis* (11.80 ± 0.84). On the other hand, chloroform extract of leaf showed high activity against *Bacillus subtilis* (13.20 ± 0.84), and chloroform extract of stem bark showed significant activity against *Staphylococcos aureus* (13.60 ± 0.89), *Bacillus subtilis*(13.20 ± 0.84) *and Klebseilla pneumoniae*(12.0 ± 1.0). Interestingly, ethanol extract of leaf exhibits maximum inhibitory activity against *Klebseilla pneumoniae* (16.40 ± 0.80) in comparison to other extracts, and ethanol extract of stem bark exhibited significant high inhibitory zones against *Bacillus subtilis* (19.20 ± 1.52), *Escherichia coli* (16.40 ± 0.55), *Staphylococcus aureus* (15.20 ± 0.84), *Klebseilla pneumoniae* (14.80 ± 1.30) and *Salmonella typhi* (13.80 ± 0.45) in comparison to other extracts. It is understandable that ethanol extract of stem bark is more potent showing a higher degree of antimicrobial activity to tested clinical bacterial pathogens in comparison to other extracts. In addition, moderate effects were seen in petroleum ether and chloroform extracts of stem bark, and chloroform

Table 2: Minimum inhibitory concentration (MIC) of various solvent extracts *L. glutinosa* against microorganisms

Test organism	Plant extracts					
	Stem bark			Leaf		
	P.E	C.E	E.E	P.E	C.E	E.E
	Concentration (mg/ml) showing Minimum inhibitor concentration					
Staphylococcus aureus	10	2.5	2.5	*	*	2.5
Bacillus subtilis	5	2.5	2.5	20	5	5
Escherichia coli	20	10	2.5	*	*	5
Pseudomonas aeruginosa	20	*	5	20	10	20
Klebsiella pneumoniae	10	*	2.5	*	10	5
Salmonella typhi	10	10	2.5	10	20	10
Salmonella paratyphi	*	10	5	*	20	5
Proteus sp.	20	20	5	*	20	5

*No inhibition observed upto: 40 mg/ml.

Table 3: Antibacterial activity screening of *L. glutinosa* extracts of Stem bark and Leaf

Bacterial cultures	Ciprofloxacin	10% DMSO	Zone of inhibition (mm diameter)					
			Stem bark			Leaf		
			P.E	C.E	E.E	P.E	C.E	E.E
Staphylococcus aureus	25.0 ± 1.22	–	5.80 ± 0.45	13.60 ± 0.89	15.20 ± 0.84	–	–	7.6 ± 0.55
Bacillus subtilis	29.40 ± 1.14	–	11.80 ± 0.84	13.20 ± 0.84	19.20 ± 1.52	2.50 ± 0.58	13.20 ± 0.84	14.0 ± 1.0
Escherichia coli	25.20 ± 0.84	–	2.40 ± 0.55	5.80 ± 0.84	16.40 ± 0.55	–	–	9.6 ± 0.89
Pseudomonas aeruginosa	29.60 ± 1.14	–	2.60 ± 0.55	–	9.80 ± 0.84	2.25 ± 0.50	4.60 ± 1.14	2.4 ± 0.5
Klebsiella pneumoniae	28.80 ± 0.84	–	6.20 ± 0.84	12.0 ± 1.0	14.80 ± 1.30	–	4.40 ± 0.55	16.40 ± 0.80
Salmonella typhi	25.0 ± 0.84	–	4.40 ± 0.55	6.20 ± 0.45	13.80 ± 0.45	5.75 ± 0.50	2.40 ± 0.55	9.80 ± 0.84
Salmonella paratyphi	23.0 ± 0.84	–	–	6.20 ± 0.84	10.0 ± 1.0	–	2.20 ± 0.84	4.60 ± 0.89
Proteus sp.	24.80 ± 0.84	–	1.40 ± 0.55	2.40 ± 0.55	10.0 ± 1.0	–	2.20 ± 0.45	5.40 ± 0.89

± - mean standard deviation of triplicates, Concentration of extract - 5 mg/disk, (–) - No zone of inhibition observed

extracts leaf against tested bacterial strains except petroleum ether of leaf which showed comparatively minimum area of inhibition. This possibly means that the compound responsible for the antibacterial activity was least in concentration.

In susceptibility test, the ethanol extracts of stem bark and leaf showed the considerable levels of inhibition against bacterial pathogens. The clinical bacterial pathogens test of petroleum ether extract showed low inhibition range in comparison to chloroform and ethanol extracts. In conjugation with phytochemical screening of all the extracts with petroleum ether, showed the absence of alkaloids derivates. Hence, it is suggested that absence of alkaloids in petroleum ether may be the cause of decreased activity in clinical bacterial pathogens. The ethanol extracts showed high inhibition at the minimal concentration for most of the clinical pathogens in comparison to other extracts. The MIC value of ethanol extract of stem bark ranges from 2.5 to 5 mg/ml for *Escherichia coli, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi, Proteus sp. Klebseilla pneumoniae, Staphylococcus aureosa* and *Pseudomonas aeruginosa.* Also, the phytochemical screening of ethanol extract of stem bark showed the presence of most of the derivatives like flavonoids, alkaloids, saponins and tannins. Furthermore, alkaloids[20,21] flavonoids,[22-24] tannins,[25-27] saponins[28] of various plants extracts proven to be effective antimicrobials.[2] Our results are also in agreement with these studies suggesting the efficacy of ethanol extract of stem bark of *L.glutinosa* against clinical pathogens.

CONCLUSION

Extracts of *L.glutinosa* showed the broad spectrum of antibacterial activity on the tested microorganisms. Ethanol extract of leaf exhibited high inhibitory potency against *Klebsiella pneumoniae* and ethanol extract of stem bark showed maximum inhibition against all tested clinical pathogens. The phytochemical analysis showed the presence of effective biological compounds like alkaloids, flavonoids, tannins and saponins. These derivatives could be potential alternatives to the traditional chemical control of clinical pathogenic bacteria. Fractionation and characterization of these active compounds will be the future work to investigate.

REFERENCES

1. Bassam AS, Ghaleb A, Naser J, Awni AH, Kamel A. Antibacterial activity of four Plant extracts used in palestine in folkloric medicine against methicillin-resistant *Staphylococcus aureus*. Turkish Journal of Biology. 2006; 30:195-8.
2. Cowan MM: Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999; 12:51-59.
3. Meenakshi Singh, Sayyada Khatoon, Shweta Singh, Vivek Kumar, Ajay Kumar Singh Rawat, Shanta Mehrotra. Antimicrobial screening of ethnobotanically important stem bark of medicinal plants. Phcog Res. 2010; 2(4):254-7.
4. Iqbal A, Arina ZB. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001; 74:113-23.
5. Piddock LJV, Wise R. Mechanisms of resistance to quinolones and clinical perspectives. J Antimicrob Chemother .1989; 23(4):475-80.
6. Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin microbiol Rev. 1993; 6:428-42.
7. Maleki S, Seyyednejad SM, Damabi NM, Motamedi H. Antibacterial activity of the fruits of Iranian *Torilis leptophylla* against some clinical pathogens. Pak J Biol sci. 2008; 11:1286-89.
8. Annan Kofi, Gbedema Stephen, Adu Francis. Antibacterial and radical scavenging activity of fatty acids from *Paullinia pinnata* L. Phcog Mag. 2010; 5(19):119-23.
9. Basile A, Giordano S, Lopez-Saez JA, Cobianchi RC. Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry. 1999; 52: 1479-82.
10. Singh B, Bhat TK. Potential therapeutic applications of some anti-nutritional plant secondary metabolites. J Agric Food Chem. 2003; 51:5579-97.
11. Hostettmann K. and Hamburger M. Antimicrobial effect of some native plants. Phytochem. 1991; 30:3864-74.
12. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses, Asiatic society of Bangladesh, Nimtali, Dhaka-1000; 2003;285.

13. Rout SD and Thatoi HN. Ethnomedicinal practices of Kol tribes in Similipal Biosphere Reserve, Orissa, India. *Ethnobotanical Leaflets.* 2009; 13:379-87.
14. Kirtikar, K. and B. Basu. Indian medicinal plants (revised issue), vol. III, Allahabad; 1981. P. 2156-61.
15. Chatterjee, A. and S. Pakrashi. the treatise of Indian Medicinal Plants. vol. I, Insa, New delhi, India; 1994.P. 107.
16. Jing-Hua Yang, Liang Li, Yun-Song Wang, Jing-Feng Zhai, Hong-Bin Zhang and Shi-De Luo. Two new aporphine alkaloids from Litsea glutinosa.Helvetica chimca ata. 2005; 88:2523-26.
17. Cowan ST, Steel S. *Manual for the identification of medical bacteria*, Barrow GI, Feltham RKA, Editors. Cambridge University press; 1993. P.32.
18. National committee for clinical laboratory standards (NCCLS): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically approved standard - NCCLS document M7-A6, Wayne, PA, USA, sixth 2003.
19. Clinical and laboratory standards institute (CLSI): Method for dilution antimicrobial susceptibility tests for bacterial that grow aerobically; approved standard – eighth edition. CLSI document M07-A8, Wayne, PA, USA 2009.
20. Shaheen F, Khan RA, Soobia A, Khan SA, Saima T, Aqeel A. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. pendula. Planta medica. 2003;69:350-355.
21. Chakraborty A, Brantner AH. Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. villosum leaves. J Ethnopharmacol. 1999; 68:339-44.
22. Brandao MGL, Krettli AU, Soares LSR, Nery CGC, Marinuzzi HC. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other Bidens species (Asteraceae) correlated with the presence of acetylene and flavonoids compounds. J Ethnopharmacol. 1997; 57:131-38.
23. Hernandez NE, Tereschuk ML, Abdala LR. Antimicrobial activity of flavonoids in medicinal plants from Tafi Del Valle (Tucuman, Argentina). J Ethnopharmacol. 2000; 73:317-22.
24. Mendoza L, Wilkens M, Urzua A. Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). J Ethnopharmacol.1997; 58:85-8.
25. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001; 48:487-91.
26. Doss A, Mubarack MH, Dhanabalan R. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian J Sci Technol. 2009; 2:41-43.
27. Amarowicz R, Dykes GA, Pegg RB. Antibacterial activity of tannin constituents from *Phaseolus vulgaris, Fagoypyrum esculentum, Corylus avellana and Juglans nigra.* Fitoterapia. 2008; 79:217-19.
28. Farnsworth, N. Biological and Physiochemical screening of plants. J. Pharmaceutical Sci. 1966; 55:225.

Antibacterial activity of *Diplazium esculentum* (Retz.) Sw

Semwal Amit[1*], Kaushik Sunil[1], Bhatt SP[1], Negi Arvind[2]

[1]Gyani Inder Singh Institute of Professional Studies, Dehradun, Uttarakhand-248001. [2]GRD (PG) IMT, Rajpur Dehradun, Uttarakhand-248001.*

ABSTRACT

The main objective of this research work is to screen various unexploited plants for their antimicrobial activity as these unexploited or pteridophytic plants are being used ethanomedicinally but, very little work has been done on antimicrobial aspects. So, to explore the efficacy of these plants, the following research has been carried out. Aqueous and alcoholic extracts of the plant parts of *Diplazium esculentum* (Retz.) Sw were tested against the growth of some human and plant pathogenic bacteria like *E.coli, Salmonella arizonae, Salmonella typhi, Staphylococcus aureus.* Near all the extracts were found effective against these bacteria. The positive results so obtained were compared with that of the Tetracycline reference standard antibiotic. It was found that extracts when mixed in equal proportion with the antibiotic were more effective against bacteria than the antibiotic alone.

Key words: Antibacterial activity, *Diplazium esculentum*, Pteridophytes.

INTRODUCTION

Pteridophytes (ferns and fern-allies) have drawn attention of plants lovers and horticulturists since antiquity. They are represented by about 305 genera and more than 1,000 species have been reported from India. [1] However, a perusal of literature reveals that more than 200 species of pteridophytes are being used by the tribals of different regions of India for the treatment of various ailments like cancer, rheumatism, diabetes, inflammation, convulsant, fertility, diuretic, anthelmintic, aphrodisiac, hepatoprotective, sedative, antipyretic etc. and their antimicrobial properties have been reported. [2-3] The screening and scientific evaluation of plant extract for their antimicrobial substance may prove beneficial for the mankind. In the present investigation an attempt has been made to test *in vitro* antibacterial activity of *Diplazium esculentum* found in Garhwal region against some human and plant pathogenic bacteria like *E.coli*, *Salmonella arizonae, Salmonella typhi, Staphylococcus aureus. Diplazium esculantum* (Retz.) Sw (Family- Athyriaceae) has creeping and branched rhizome; scales brown, lanceolate and upto 7 to 15 mm in length; stripes fragile, straw coloured, 10-35 cm long, lamina variable, broadly lanceolate to sub-deltoid with acuminate apex, decompoundly pinnae and stalked, distantly placed, 6-15 × 2-4.7 cm ascending with slender, naked, greenish rachides, pinnules, upto 15 × 6 mm, sub deltoid, cut down into ultimate oblong, narrow segments with dentate margin, secondary rachides minutely pubescent, veins forked; sori minute, indusium thin, membranous; spores dark brown.

The plant is common in Uttarakhand region and is frequently used by the Garhwali peoples for medical purpose. The circinately coiled young leaves are used as vegetables (lingra). The rhizome is considered as strong anthelmintic, haemoptysis, used in cough, asthma, phthisis, fever, dyspepsia, stomachache, diarrhea and as antidysenteric, insect and pest repellant. Young tips of fronds are used as tonics for health. Decoction of rhizome and young leaves are useful haemoptysis and constipation.[4-8]

***Address for correspondence:**
Mobile:09453036379
E-mail: semwal_amit@rediffmail.com

DOI: 10.5530/pj.2011.21.14

MATERIALS AND METHODS

The specimens of plant were collected from Shyampur, Rishikesh in Uttarakhand region during the month of August and their identity was confirmed by Botanical Survey of India (BSI), Dehradun.

Preparation of Plant Extracts

Fresh plants parts (5 g) were washed 2-3 times with tap water and distilled water and then surface sterilized with 90% alcohol. Subsequently, the plant material was grounded in 50 ml of distilled water and acetone separately for aqueous and alcoholic extracts respectively. The alcoholic macerates were kept for 24 hours at room temperature to evaporate the alcohol. In the remaining residue, 50 ml of distilled

water was added. Macerates were squeezed through double layer muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10000 rpm for 20 minutes. The supernatants were filtered through Whatman filter paper and then sterilized by passing through 0.2-micron disposable filters. The extracts (10%) thus obtained were used for the in vitro studies. [9]

Antibacterial activity of plant extracts

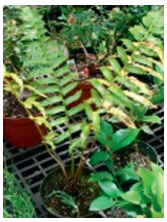
The bacterial cultures were obtained from the GISIPS, Dehradun and maintained on the nutrient agar. The disc diffusion method was used for testing anti bacterial activity.[10] The media (25 ml) inoculated with suspension of experimental organism was poured in to sterilized petri dishes and left to gel at room temperature. Whatmans filter paper discs (7 mm diameter) were soaked in 0.2 ml aqueous and alcoholic extracts as well as a 10-ppm solution of Tetracycline. The filter discs were placed equidistantly on inoculated media and diffusion of solution were allowed to occur for 30 minutes at room temperature. Petri dishes were incubated at 37°C for 24 hours. Three plates were employed per treatment and the average zone of inhibition was recorded.

RESULT AND DISCUSSION

The rhizome and root extracts inhibited the micro organism growth were as the leaves extract did not show any inhibition. It was observed that the root extract along with antibiotic showed maximum inhibition against *Salmonella arizonae* than the reference standard alone. The leaves and roots extracts did not show any inhibition against *E.coli*. The aqueous extract of root along with antibiotic has shown higher inhibition against *Staphylococcus aureus* than the antibiotic alone. The rhizome extracts were found to be more effective than antibiotic.

Leaflet of D. esculentum

Frond of D. escule ntum



Plant of D. esculantum

CONCLUSION

It is concluded that antibacterial activity of *Diplazium esculentum* and its active constituents would be helpful in treating various kinds of diseases. Crude extracts and their interaction with different active fractions of the plant are needed to explore the exact mechanism of the interaction among the active phyto constituents. Similarly, the efficacy of crude extracts or polyherbal preparation needed to be studied in vitro to assess their therapeutic utility.

ACKNOWLEDGEMENT

Authors are thankful to Prof. A.K.Wahi, Director, GISIPS, Microbiology Laboratory, Dehradun for providing laboratory facilities and for supplying bacterial cultures.

REFERENCES

1. Dixit RD, Fern- A Much Neglected Group of Medicinal Plants. III, J Res Indian Med. 1984; 19:309-314.
2. Sharma BD, Vyas MS, Ethnobotanical Studies on the Ferns and Fern allies of Rajasthan, Bull Bot Surv India, 1985; 27(1-4):90-91.
3. Singh M , Singh N, Khare PB, Rawat AK, **Title missing** J Ethnopharmacol. 2008; Jan, 17,115(2):327-9.
4. Singh HB, Potential medicinal Pteridophytes of India and their chemical constituents, J. Econ. Taxon Bot, 1999; 23 No.1:63-78.
5. Bir SS, Key notes address on Ferns of India, their wealth exploration, diversity, growth conditions and conservation, Indian Fern J, 1992; 9:IV-VI.
6. Kaushik P, Dhiman A.K., Common Medicinal Pteridophytes, Indian ferns J., 1995; 12:139-145.

Table 1: Antibacterial activity of plant part extracts of Diplazium esculentum by disc diffusion method.

Plant parts	Extracts		Diameter of Inhibition Zone (in mm)			
			E.coli	*Salmonella arizonae*	*Salmonella typhi*	*Staphylococcus aureus*
Leaves	Extract	Aqueous	00	07	02	00
		Alcoholic	00	09	00	00
	Extract + Antibiotic	Aqueous	20	23	24	24
		Alcoholic	19	19	22	21
Rhizome	Extract	Aqueous	15	05	12	10
		Alcoholic	18	10	11	10
	Extract + Antibiotic	Aqueous	20	18	25	26
		Alcoholic	18	24	24	22
Roots	Extract	Aqueous	10	10	15	15
		Alcoholic	11	12	10	14
	Extract + Antibiotic	Aqueous	22	20	24	24
		Alcoholic	18	20	22	34
Tetracycline (Reference Standard Antibiotic)			22	24	28	24

7. Quisumbing E, Medicinal Plants of Philippines, Manila Dept. Arg. Nat. Res. Bull., 1951; 16(2):174-176.
8. Singh S, Dixit RD, Sahu TR, Some Medicinally Important Pteridophytes of Central India, Int.J. For. Usuf. Magt., 2003; 4(2):41-50.
9. Parihar P, Bohra A, Antisalmonellae activity of three species of Adiatum found in Rajasthan, Indian Fern J, 2004; 21:136-139.
10. Nene YL, Thapliyal PN, Fungicides in plants disease control, Oxford and IBH Publishing Co., 1979; 415-416.

Antimicrobial Activity of Six Different Parts of the Plant *Citrus medica* Linn.

Archana N. Sah*, Vijay Juyal[1], Anand B. Melkani[2]

Department of Pharmaceutical Sciences, Faculty of Technology, Kumaun University Nainital, Uttarakhand, 263136. [1]Uttarakhand Technical University, Dehradun, Uttarakhand, [2]Department of Chemistry, Kumaun University, D. S. B. Campus Nainital, Uttarakhand, India.

ABSTRACT

Introduction: Antimicrobial activity of fruit juice and ethanolic extracts of root, leaf, bark, peel and pulp of citron (*Citrus medica* Linn., Rutaceae) was examined against seven bacteria (*Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus vulgaris*), two fungi (*Aspergillus flavus* and *A. niger*) and a yeast *Candida albicans* of clinical origin. **Methods:** The level of antimicrobial effects was established using an *in vitro* disc diffusion method; minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined by standard agar dilution method. **Results:** All extracts and fruit juice showed varied level of antibacterial activity against one or more test bacteria. Antifungal activity was shown by only root extract and fruit juice while *C. albicans* was resistant to all tested plant samples. Broad spectrum antimicrobial activity was shown by fruit juice (MIC <1% to 3.5% and MBC 1% to 7% v/v) and fruit pulp (MIC 25 mg/ml and MBC 30 to 75 mg/ml). Root extract was found highly potent with MIC as small as 0.5 mg/ml and MBC 1 mg/ml against *S. aureus*. Among all tested plant samples leaf and peel extracts have shown less antimicrobial activity. **Conclusion:** It is concluded that fruit juice and juiceless fruit pulp extract have shown broad antimicrobial activity while root extract was very effective against some tested microorganisms.

Key words: Antibacterial, antifungal, antimicrobial activity, citron, *Citrus medica* Linn.

INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world.[1-3] However, the situation is critical in developing and developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients.[4] The public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. It is reported that on average, two or three antibiotics derived from microorganisms are launched each year.[5] Scientists realize that due to resistance of microorganisms the effective life span of any antibiotic is limited.[6] In the present scenario of emergence of multiple drug resistance to human pathogens, this has necessitated to search new antimicrobial substances from other sources including plants.

The substances that can inhibit the growth of microorganisms or kill them and have little or no toxicity to human cells are considered candidates for developing new antimicrobial drugs. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties.[7-9] In recent years antimicrobial properties of medicinal plants have been increasingly reported worldwide.[10-17] In the present study, we have selected *Citrus medica* Linn. commonly known as citron; which was not previously screened for antimicrobial properties. However, most of the *Citrus* species were previously screened against pathogenic organisms.[18-22] Many parts of this plant are used in the Indian traditional system of medicine against various ailments. Ripe fruits are used in sore throat, cough, asthma, thirst, hiccough, earache, vomiting; potent antiscorbutic, stomachic, tonic, stimulant, expellant of poison, correct fetid breath; distilled water of the fruit is sedative; fruits and seeds are cardiac tonic and useful in palpitation; fruit decoction is analgesic. Roots, flowers, seeds, peels and leaves are also used in many ailments.[23,24] Fruit extracts have also shown good antioxidant

Address for correspondence:
Tel: +91-05942-247030, +91-9410566599,
Fax: +91-05942-248307
E-mail: archanansah@gmail.com

DOI: 10.5530/pj.2011.21.15

activity.[25] The fruit wrapped in cloth is used to protect clothes from moth indicates its insect-repellent activity.[26] In ancient literature citron was mentioned as an antidote of every kind of poison.[27]

It was expected that screening of fruit juice and extracts of root, leaf, bark, peel and pulp against wide variety of microorganisms would be helpful in obtaining broad-spectrum new antimicrobial substances. The aim of this study was to test plant extracts against a diverse range of organisms including gram-positive and gram-negative bacteria, two fungi and a yeast.

MATERIALS AND METHODS

Plant material

All samples of *C. medica* Linn. were collected locally. The plant was identified as *C. medica* Linn. by an authorized taxonomist at National Botanical Research Institute, Lucknow. Voucher specimen number 97840 has been deposited in the same institute.

Preparation of plant extracts

Fresh leaves and peels were shade dried and powdered. Bark and root samples were sun dried and powdered. Ripe fruits after removing peels were squeezed to collect juice and stored at 4-6ºC until used. Juiceless pulps were dried under sun and powdered. Extracts of bark and pulp were prepared by soaking 100 gm of powdered samples in 250 ml of 70% ethanol while 100 gm of root, leaf and peel samples were soaked in 500 ml of 70% ethanol for 96 h. Each mixture was stirred every 24 h using sterile glass rod. At the end of extraction each extract was passed through Whatman filter paper no. 1 (Whatman, UK). The filtrates were concentrated on a rotary evaporator at 40ºC, dried and weighed. The percentage of extract with respect to crude drug was found as 7.43% (root), 9.6% (leaf), 5.8 % (bark), 24.6% (peel) and 23.26% (pulp).

Microorganisms used

Microorganisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. Bacterial cultures were *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 737, *Enterococcus faecalis* MTCC 439, *Escherichia coli* MTCC 1687, *Klebsiella pneumoniae* MTCC 109, *Pseudomonas aeruginosa* MTCC 1688 and *Proteus vulgaris* MTCC 1771. The filamentous fungi used were *Aspergillus flavus* MTCC 277 and *Aspergillus niger* MTCC 1344; and a yeast *Candida albicans* MTCC 227. All cultures were Micrbial Type Culture Collection (MTCC).

Culture media and inoculum

The fungi were maintained on Sabouraud Dextrose Agar (SDA; Hi-Media, India) and bacteria on Nutrient Agar (NA; Hi-Media, India) at 4ºC temperature until used in the study. Before use, the fungal cultures were revived in Sabouraud Dextrose Broth (SDB; Hi-Media, India) and bacterial cultures on Nutrient Broth (NB; Hi-Media, India). Inoculated broths were incubated overnight at 37ºC for bacteria and at 28ºC for fungi.[28] The freshly grown microbial cultures were appropriately diluted in sterile broth media to obtain the cell suspension of 10^6 cfu/ml.

Antimicrobial assay

The agar disc diffusion method[29] was used; 0.1 ml of diluted inoculum (10^6 cfu/ml) of test organism was spreaded on SDA/NA plates. Sterilized Whatman papers discs (6 mm diameter) were soaked in fruit juice and solutions of extracts (100 or 200 mg/ml) in dimethyl sulfoxide (DMSO, Sigma) and solvent blank (DMSO). Chloramphenicol discs (25 µg/disc; CDH, Central Drug House, New Delhi) were used in the test system as positive control against test bacteria while itraconazole against test fungi and yeast. The bacterial plates were incubated for 24 h at 37ºC while fungal and yeast plates were incubated for 48 h at 28ºC. The antimicrobial activity was evaluated by measuring the zone of inhibition (ZOI) against test organisms. Experiments were performed in triplicate.

MIC and MBC determinations

Agar dilution method[30] was adopted to find minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of active extracts. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and MBC as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antimicrobial-free media.[31]

RESULTS

Antimicrobial activity of six different extracts of *C. medica* Linn. is shown in Table 1 in terms of zone of inhibition. DMSO as control did not show any inhibition against all tested microorganisms. All extracts failed to inhibit growth of *C. albicans*. Fruit juice inhibited all tested Gram-positive, Gram-negative bacteria and two fungi *A. niger* and *A. flavus* with zone of inhibition comparable to standard drugs. Semidried extract of juiceless fruit pulp have also shown similar results as fruit juice except against *B. subtilis, A. niger* and *A. flavus*. Only difference was in the zone size which was less in case of fruit pulp than juice.

Both fungi *A. niger* and *A. flavus* were inhibited by root extract and fruit juice. Root, leaf and bark extracts inhibited *S. aureus, E. faecalis* and *P. vulgaris* with maximum inhibition by root extract comparable to standard antibiotic. Fruit

Table 1: Antimicrobial properties of *C. medica* Linn.

Microorganisms (MTCC code)	Zone of inhibition in mm						
	Plant part used						Standard drug (25 µg/disc)
	Root	Leaf	Bark	F. peel	F. pulp	F. juice	
Gram positive bacteria							
Bacillus subtilis 441	–	–	–	–	–	16	23
Staphylococcus aureus 737	23	10	11	08	13	14	28
Enterococcus faecalis 439	13	13	13	–	15	16	24
Gram negative bacteria							
Escherichia coli 1687	–	–	–	–	12	16	28
Klebsiella pneumoniae 109	–	–	–	08	13	18	27
Pseudomonas aeruginosa 1688	–	–	–	–	12	19	28
Proteus vulgaris 1771	19	09	12	10	13	17	14
Fungi							
Aspergillus niger 1344	10	–	–	–	–	12	20
Aspergillus flavus 277	12	–	–	–	–	10	21
Yeast							
Candida albicans 227	–	–	–	–	–	–	23

–, no inhibition; NT, not tested; MTCC, microbial type culture collection; F, fruit; diameter of disc 6mm; extract conc 200 µg/ml; results are average of three readings. Chloramphenicol was used as standard against bacteria and Itraconazole as antifungal and anticandidal.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data of *C. medica* Linn. extracts obtained by the agar dilution method

Microorganisms (MTCC code)	Plant part used									
	Root		Leaf		Bark		F. pulp		F. juice	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. subtilis 441	–	–	–	–	–	–	–	–	2.5	3.5
S. aureus 737	0.5	1	10	25	25	50	25	30	2.0	3.0
E. faecalis 439	>15	>15	45	50	50	75	25	75	2.5	7.0
E. coli 1687	–	–	–	–	–	–	25	30	2.0	3.0
K. pneumoniae 109	–	–	–	–	–	–	25	30	3.5	3.5
P. aeruginosa 1688	–	–	–	–	–	–	25	30	3.0	6.0
P. vulgaris 1771	5	15	10	10	25	50	25	30	<1.0	1.0

–, not tested; MTCC, microbial type culture collection; F, fruit; values of MIC and MBC are given as % of juice and mg/ml of extracts.

peels have shown least activity among all extracts and slightly inhibited growth of *S. aureus, K. pneumoniae* and *P. vulgaris*. The yeast *C. albicans* was not inhibited by any extract. Among bacteria *S. aureus* and and *P. vulgaris* were highly susceptible to all extracts while *B. subtilis* was highly resistant and inhibited by only fruit juice.

Table 2 shows MIC and MBC of active extracts against susceptible microorganisms. Root extract had the lowest MIC 0.5 mg/ml and MBC 1 mg/ml against *S. aureus*. The maximum MIC of extracts was 50 mg/ml and MBC 75 mg/ml. The minimum MIC of juice was <1% and MBC 1% against *P. vulgaris* while maximum MIC was 3.5% and MBC 7%.

DISCUSSION

Various publications have documented the antimicrobial activity of plant extracts and essential oils including lemon juice against HIV, syphilis, gonorrhea,[32] *Vibrio cholera*[33] and topical microbicide.[34] Published results on the antimicrobial activity of *Citrus* extracts suggested screening of such activity of *C. medica* Linn. variety available in Kumaun region of Uttarakhand, India which was not previously screened for antimicrobial activity.

In the present study fruit juice was found to be the most effective agent against all tested bacteria and fungi; juiceless pulp was the next effective extract. The basis of varying degree of sensitivity of test organisms may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the crude extract. One or more of the common phytoconstituents like alkaloids, tannins, phenols, glycosides, flavonoids and acids were already reported in some of these active extracts.[35,36] These major phytocompounds are known to have antimicrobial activity.[32]

On the basis of the present investigation it can be highlighted that some of the extracts of *C. medica* Linn. showed promising antibacterial and antifungal properties and could

be exploited in herbal preparations for both external and internal uses. Since the fruit is edible and used by the people of Kumaun in the preparation of pickles, juices and other recepies so can be used in herbal preparations for internal use also. Root is also reported in ancient Indian literature to be used as anthelmintic and in urinary calculus, so it can also be used internally.[23]

CONCLUSION

The active extracts can also be screened against more human pathogens including human immunodeficiency virus (HIV) and antibiotic resistant strains. The active principles of these extracts are required to be isolated, characterized and tested for their safety and efficacy to uncover their therapeutic potential in modern medicine against infectious diseases. Furthermore of these compounds can be subjected to animal and human studies to determine their effectiveness in whole organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal micro biota.

ACKNOWLEDGEMENTS

The authors wish to thank Head, Department of Pharmaceutical Sciences, Faculty of Technology, Kumaun University Nainital, Uttarakhand, India for providing necessary facilities to complete this work.

REFERENCES

1. Davis M. Inactivation of antibiotic and the dissemination of resistance genes. Science. 1994; 264:375-82.
2. Robin EH, Anril W, Alexander M, Loeto M, Keith K. Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and *Heamophilus influenzae* Tupe b in children under 5 years of age in Botswana. I J Infect Dis. 1998; 3:18-25.
3. Zampini IC, Cuello S, Alberto MR, Ordonez RM, Almeida RD, Solorzano E, et al. Antimicrobial activity of selected plant species from "The Argentine Puna" against sensitive and multi-resistant bacteria. J Ethnopharmacol. 2009; 124:499-505.
4. Diamond RD. The growing problem of mycoses in patients infested with human immunodeficiency virus. Rev Infect Dis. 1993; 13:480-6.
5. Clark AM. Natural products as a resource for new drugs. Pharm Res. 1996; 13:1133-41.
6. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999; 12:564-82.
7. Iyengar MA. Study of crude drugs. 13th ed. College of pharmaceutical sciences Manipal: India; 1985.
8. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian medicinal plants. Council of Scientific and Industrial Research: New Delhi; 1992.
9. Harborne SB, Baxter H. Phytochemical dictionary: A handbook of bioactive compounds from plants. London: Taylor and Francis; 1995.
10. Grosvenor PW, Supriono A, Gray DO. Medicinal plants from Riau Province, Sumatra, Indonesia: antibacterial and antifungal activity. J Ethnopharmacol. 1995; 45:97-111.
11. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999; 86:985-90.
12. Saxena VK, Sharma RN. Antimicrobial activity of essential oil of *Lantana aculeata*. Fitoterapia. 1999; 70:59-60.
13. Dulger B, Gonuz A. Antimicrobial activity of some endemic *Verbascum, Salvia,* and *Stachys* species. Pharm Biol. 2004; 42:301-4.
14. Dutta BK, Karmakar S, Naglot A, Aich JC, Begam M. Anticandidal activity of some essential oils of a mega biodiversity hotspot in India. Mycoses. 2007; 50:221-4.
15. Pirbalouti AG, Malekpoor F, Enteshari S, Yuosefi M. Antibacterial activity of some folklore medicinal plants used by Bakhtiari tribal in Southwest Iran. I J Biol. 2010; 2:55-63.
16. Mariita RM, Orodho JA, Okemo PO, Mbugua PK. Antifungal, antibacterial and antimycobacterial activity of *Eutada abysinnica* Steudel ex A. Rich (Fabaceae) methanol extract. Phcog Res. 2010; 2(3), 163-8.
17. Arranz JCE, Roses RP, Laffita IU, Pozo MIC, Amado JR, Jimenez IL. Antimicrobial activity of extracts from *Tamarindus indiaca* L. leaves. Phcog Mag. 2010; 6(23), 242-7.
18. Cvetnic Z, Knezevic SV. Antimicrobial activity of grapefruit seed and pulp ethanolic extract. Acta Pharm. 2004; 54:243-50.
19. Fisher K, Philips CA. The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni, Escherichia coli* 0157, *Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. J Appl Microbiol. 2006; 101:1232-40.
20. Conte A, Speranza B, Sinigaglia M, Del Nobile MA. Effect of lemon extract on foodborne microorganisms. J Food Prot.2007; 70(8):1896-900.
21. Kisla D. Effectiveness of lemon juice in the elimination of *Salmonella typhimurium* in stuffed messels. J Food Prot. 2007; 70(12):2847-50.
22. Sanguinetti M, Posteraro B, Romano L, Battaglia F, Lopizzo T, De Carolis E, et al. In vitro activity of *Citrus bergamia* (Bergamot) oil against clinical isolates of dermatophytes. J Antimicrob Chemother. 2007; 59(2):305-8.
23. Kirtikar KR, Basu BD. Indian Medicinal Plants. Dehradun: Bishan Pal Singh Mahendra Pal Singh Publications, p. 485-90; 1993.
24. Nadkarni AK. Indian materia medica. Bombay: Popular Prakashan, p. 348; 1996.
25. Jayaprakash GK, Patil BS. In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange, Food Chem. 2007; 101:410-18.
26. Singh VK, Ali ZA. Herbal drugs of Himalaya. New Delhi: Today and Tomorrow's Printers and Publishers, p. 70; 1998.
27. Beatriz AA, Luis RL. Pharmacological properties of *Citrus* and their ancient and medieval uses in the Mediterranean region. J Ethnopharmacol. 2005; 97:89-95.
28. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, Editors. Practical medical microbiology. New York: Churchill Livingstone, p.116; 1996.
29. More G, Tshikalange TE, Lall N, Botha F, Meyer JJM. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol. 2008; 119:473-7.
30. Miles RS, Amyes SBG. Laboratory control of antimicrobial therapy. In: Collee JG, Fraser AG, Marmion BP, Simmons A, Editors. Practical medical microbiology. New York: Churchill Livingstone, p. 151-78; 1996.
31. Jennifer MA. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48:5-16.
32. Bruneton J. Pharmacognosy and phytochemistry of medicinal plants. France: Lavoisler, p. 265-380; 1995.
33. De Castillo MC, Allori CG, Gutierrez RC, Saab OA, Fernandez NP, Ruiz CS, et al. Bactericidal activity of lemon juice and lemon derivatives against *Vibrio cholerae*. Biol Pharm Bull. 2000; 23:1235-8.
34. Fletcher PS, Harman SJ, Boothe AR, Doncel GF, Shattock RJ. Preclinical evaluation of lime juice as a topical microbicide candidate. Retrovirol. 2008 Jan 11; 5(3).
35. Albach RF, Redman GH. Composition and inheritance of flavanones in *Citrus* fruit, Phytochem.1969; 8:127-43.
36. Khare CP. Indian medicinal plants. New Delhi: Springer, p. 156; 2007.

Coriandrum sativum: A Daily Use Spice with Great Medicinal Effect

Poonam Mahendra,* Shradha Bisht

Department of Pharmacology, School of Pharmacy, Suresh Gyan Vihar University, Jaipur 302004, India

ABSTRACT

Coriandrum sativum Linn. has been credited with many medicinal properties. The green leaves of coriander are known as "asotu" in the Eastern Anatolian region or "cilantro" in the United States and are consumed as fresh herb. The essential oil obtained from its fruits at amounts ranging from approximately 0.5 to 2.5% is used both in flavours and in the manufacture of perfumes and soaps. The plant is grown widely all over the world for seed, as a spice, or for essential oil production. It is one of the earliest spices used by mankind. It has been used as a flavouring agent in food products, perfumes and cosmetics. It is used for various purposes such as for flavouring sweets, beverages, tobacco products and baked goods and as a basic ingredient for curry powder. It has been used as an analgesic, carminative, digestive, anti-rheumatic and antispasmodic agent.

Key words: *Coriandrum sativum Linn*, essential oil, flavouring agent.

INTRODUCTION

Coriander (*Coriandrum sativum L*) is an annual and herbaceous plant, belonging to the Apiaceae family (carrot family). Its name is derived from the Greek Koris, meaning bedbug, because of the unpleasant, fetid, bug-like odour of the green herb and unripened fruits. Green coriander (also called cilantro or Chinese, Mexican or Japanese parsley) has been called the most commonly used flavouring in the world due to its usage across the Middle East into all of southern Asia as well as in most parts of Latin America. It is native of southern Europe and the western Mediterranean region. This widely used herb is cultivated worldwide.[1] Today it is grown extensively in India, the Soviet States, central Europe, Asia, Morocco, and South and Western Australia. Its fruits (commonly called 'seeds') are used for flavouring candies, in cookery, perfumery and beverages and in the tobacco industry. *C. sativum* is approximately 30-100 cm in height, with glabrous, greatly divided, strong-smelling leaves. The odour and flavour of mature seed and fresh herbage are completely different. The mature fruits have a fresh and pleasant flavour and are largely used all over the world in ground or volatile isolate form. The composition of the essential oil of coriander fruits in some of the world has been studied and found differs from each other. The coriander plant yields two primary products which are employed for flavouring purposes: the fresh green herb and the spice (mature dried seed capsule or fruit). The odour and flavour of these two products are markedly different. In additional to its traditional use as a spice and medicinal plant, the plant has an economic importance as it is used as a flavouring agent in food products, perfumes, cosmetics and soaps.[2,3,4,5] It is widely used in India in food and as a medicine in Indian systems of medicine. It has been held in great esteem amongst indigenous medicines, particularly many medicine systems from the earliest times.

Classification

Kingdom	– Plantae
Subkingdom	– Tracheobionta
Superdivision	– Spermatophyta
Division	– Magnoliophyta –
Class	– Magnoliopsida
Subclass	– Rosidae
Order	– Apiales
Family	– Apiaceae
Genus	– Coriandrum L.
Species	– Coriandrum sativum L.

Address for correspondence:
Cell: +91 9252256367
E-mail: poonammahendra84@gmail.com

DOI: 10.5530/pj.2011.21.16

CHEMICAL CONSTITUENTS

Eighty one compounds were identified from the coriander leaf essential oil with two- dimensional gas chromatography.[6] Dried, ripe coriander fruit contain steam volatile oil, fixed (fatty) oil, proteins, cellulose, pentosans, tannins, calcium oxalate and minerals. At one time, coriander was among the world's leading essential oil plants.[7] The major constituents are fibre (23-36%), carbohydrates (about 20%), fatty oil (16-28%) and proteins (11-17%). The residues remaining after distillation of the essential oil contain high fat and protein, which is useful as animal feed. The most important constituents of coriander seeds are the essential oil and the fatty oil.[8] The chemical composition and the percentage of the components in the essential oil of the coriander fruits depend on the different stages of maturity.[4] It is reported that coriander seed oil contains linalool (60-70%) and 20% hydrocarbons and the composition of the herb oil completely differs from the seed oil. The essential oil content of the weight of ripe and a dried fruit of coriander varies between 0.03 and 2.6%, and the content of fatty oil varies between 9.9 and 27.7%. Dried coriander seeds contain as essential oil (0.03-2.6%) with linalool as the main component.[8,10,11] Other components of the essential oil are: α-thujene, sabinene, β-pinene, myrcene, p-vymene, limonene, z-β-ocimene, y-terpenin, terpinolene, camphor, citronellal, trpinene-4-ol, decanal. Cumin aldehyde, terpenene-7-al (α), terpenene-7-al (y) and geranyl acetate.[8,10,12] About 13-18% dry weight of the seed is fatty oil, of which up to 75% can be petroselenic acid which has an industrial usage as to form lauric acid in soaps and detergents and also C6 dicarboxylic acid is use as a feedstock in the manufacture of nylon.[13, 14] Other constituent of dried seeds are crude protein (11.5-21.3%), fat (17.8-19.15%), crude fiber (28.4-29.1%) and ash (4.9-6.0%)[8] Selenium contents were reported to be higher in coriander than in other herbs and herbal teas[15] The presence of minerals, such as Mg, Al. Si, P, Cl,, K Ca, Ti, Mn, Fe, Cu, and Zn were also reported[16] Anti-nutritive compounds such as glucosinolates (27.5 μmol/g), sinapine (4 mg/g), condensed tannins (1.1 mg/g) and inositol phosphates (17.4 mg/g) also present in *C. sativum* seeds.[17]

TRADITIONAL USE

At one time, coriander was among the world's leading essential oil plants[7] The traditional uses of the plant, which are based on the primary products, i.e. the fruits and the green herb, are two-fold: medicinal and culinary. *C. sativum* is a popular spice and is finely grounded to be a major ingredient of curry powder.[2] The fruits are used in the preparation of fish, meat and also for baking.[10] The seed has also been used to treat indigestion, worm infections, rheumatism, loss of appetite, convulsion, insomnia, anxiety and pain in the joints[2,4,10] Coriander is used traditionally in Morocco as a diuretic plant.[18] In Iranian folk medicine, it has been recommended for relief of anxiety and insomnia.[19] It is widely used as folk medicine as carminative, spasmolytic, digestive and galactagogue; seed extract antimicrobial; used in lotions and shampoos; with castor oil useful in rheumatism.[20-24]

PHARMACOLOGICAL USE

Antidiabetic activity

C. sativum showed significant hypoglycaemic action in rats fed with high cholesterol diet. The activity of glycogen phosphorylase and gluconeogenic enzymes revealed a decrease in the rate of glycogenolysis and glucogenesis. There was also an increased activity of glucose-6-phosphate dehydrogenase and glycolytic enzymes used glucose by the pentose phosphate pathway and glycolysis respectively.[25] In an in-vitro study to assess the possible effects of aqueous coriander plant extract (50 g plant extract/L) on glucose diffusion across the gastrointestinal tract, it was found that the extract significantly decreased glucose diffusion compared to control with mean external glucose concentration of 6.4 ± 0.2 mmol/L at 26 h. Part of the antihyperglycemic action of *C. sativum* may be due to decreased glucose absorption in vivo.[26] Pre-treatment with *C. sativum* protected Wistar albino rats against gastric mucosal damage induced by ethanol. The protective effect might be related to the free-radical scavenging property of the different antioxidant constituent present in *C. sativum*.[27] Other studies also shows that C. sativum has antidiabetic activity.[28-29]

Antioxidant activity

The ethanol extract of *C. sativum* leaves is an excellent which is stable at high temperature and can serve as a substitute for synthetic antioxidants.[30] The aqueous extract of coriander seed inhibited peroxidised lipid-induced lysis (induced by $FeSO_4$-ascorbate, 10:100 μmol/system) by 72% in human erythrocyte membranes.[31] Extract of coriander seeds obtained with supercritical carbon dioxide in semi continuous lab-scale equipment with low density (0.60 g/mL) CO_2 and high density (0.73-0.83 g/mL) CO_2 (pressure from 116 to 280 bar and temperature from 311 to 331 K for the latter) exhibited significant activity in removing free radicals present in a methanol solution of DPPH in a manner which was comparable to those of commercial antioxidants.[32]

The antioxidant capacity of phenolic compounds in coriander leaves was higher than that of the seeds in three different bioassays, namely scavenging of free radical by DPPH, inhibition of 15-lipoxygenase (15-LO) and

inhibition of Fe^{2+} induced phospholipids peroxidation in brain. The seed lipid content which was extracted with dichloromethane gave low activities in radical scavenging and inhibition of lipid peroxidation. The ethyl acetate extract of the leaves exhibited the most potent activity.[2] Assessment of the total antioxidant activity of methanol and water extracts of coriander leaves and stems using an iron-induced linoleic acid oxidation model system showed that the methanol-derived leaf extracts exhibited significantly greater radical-scavenging activity towards both lipid and water soluble radicals, which was attributed to the total phenolic content.[33] Further studies by Melo et al. indicated that the four coriander extract fraction obtained from the crude extract using chromatography in silica gel possessed similar antioxidant activities that can be measured by the β-carotene/linolic acid system. The antioxidant activity was due to several phenolic acids and caffeic acid which were contained in all four fractions.[34] Extracts of different polarity from leaves and seeds of *C.sativum* and its oil have potential as a natural antioxidant and thus inhibit unwanted oxidation processes.[35] The antioxidative effect of coriander seeds against HCH-induced formation of free radicals in rat liver.[36]

Antimutagenicity activity

Coriander played a protective role against the deleterious effects in lipid metabolism in experimental colon cancer induced by 1, 2-dimethyl hydrazine in rats.[37] The antimutagenicity of coriander juice against the mutagenic activity of 4-nitro-o-phenylenediamine, m-phenylenediamine and 2-aminofluorene was investigated using the Ames reversion mutagenesis assay with the *S. Typhimurium* TA98 strain as the indicator organism. It was found that aqueous crude coriander juice significantly decreased the mutagenicity of metabolised amines. An aqueous juice of C. sativum showed antimutagenic effect on the three tested amines, decreasing its mutagenic effect in a dose-dependent manner. In the case of 4-nitroo-phenylenediamine (NOP), an 83.21% in mutagenesis reduction was observed at the highest extract concentration, an 87.71% for m-PDA and a 92.43% for 2-AF.[38] The capacity of coriander essential oil to induce nuclear DNA damage-responsive genes was tested using suitable Lac-Z fusion strains for RNR3 and RAD51, which are genes involved in DNA metabolism and repair, respectively. At equitoxic dose, the essential oil demonstrated significant gene induction, approximately the same as that caused by hydrogen peroxide, but much lower than that caused by methyl methanesulfonate (MMS). It affected the mitochondrial structure and function and can stimulate the transcriptional expression of DNA damage-responsive genes. It appeared that the induction of microbial damage was closely linked to overall cellular cytotoxicity by the essential oil which also appeared to mask the occurrence of nuclear genetic events.[39]

Immunomodulatory activity

The aqueous crude extracts of *C. sativum* stimulated the proliferation of human peripheral blood mononuclear cells (PBMC) and the secretion of IFN-y at concentration between 50 and 200 μg/ml. Further studies on several bioactive compounds known to be of the extract, shown that flavonoids quercetin stimulated the proliferation of human PBMC and the secretion of IFN-y. However, the flavonoid rutin, coumarins bergapten and xanthotoxin modulate the secretion of IFN-y but did not enhance the proliferation of human PBMC while the coumarin isopimpinellin, promoted the proliferation of PBMC but did not modulate the secretion of IFN-y.[40, 34]

Anthelmintic activity

Crude aqueous and hydro-alcoholic extract of the seeds of *C. sativum* completely inhibited hatching of nematode eggs at concentration lower than 0.5 mg/mL with no statistically significant difference between both extracts. However, the hydroalcoholic extract showed better *in vitro* activity against adult parasites than the aqueous one. Efficacy of anthelmintic activity in vivo was tested by faecal egg count reduction (FECR) and total worm count reduction (TWCR) in sheep's artificially infected with *Haemonchus contortus*. Significantly FECR was detected on day 2 after treatment with 0.9 g/kg of crude aqueous extract of *C. sativum*. On days 7 and 14, FECR was also detected at 0.45 g/kg dose of crude aqueous extract. A significant TWCR was only detected with 0.9 g/kg dose of crude aqueous extract.[41]

Antimicrobial activity

Coriander oil strongly inhibited gram-positive bacteria (*Listeria monocytogenes and Staphylococcus aureus*) and *S. cerevisiae,* but had little effect gram-negative bacteria (*Pseudomonas fragi, Echerichia coli, Salmonella typhimurium*).[42] Methanol and water extracts of coriander leaves and stems were tested for antimicrobial activity towards *Bacillus subtills* and *Escherichia coli* by determining cell damage. The greater bacterial cell damage caused by the methanol stem extract resulted in a greater growth inhibition of the bacteria, which corresponded to the ferrous sequestering activity of the methanol-derived stem extracts.[33] The essential oil of *C. sativum* showed antimicrobial activity, varying from 125 lg/ml (C. parapsilosis CBS 604) to 500 lg/ml (*C. albicans* CBS 562), against most of the Candida species tested, except for *C. tropicalis* CBS 94.[43] This antimicrobial activity against bacteria and fungi has also been demonstrated in essential oils extracted from *C. sativum* seed.[44]

Anxiolytic effect

The aqueous extract of *C. sativum* seed has anxiolytic effect and may have potential sedative and muscle relaxation effect. The aqueous extract (100 mg/kg, i.p.) showed an anxiolytic effect in male albino mice using the elevated plus-maze

model by increasing the time spent on open arms and the percentage of open arm coordination. Furthermore, the aqueous extract (50, 100 and 500 mg/kg) significantly reduced spontaneous activity and neuromuscular coordination compared to the control group.[19]

Antidiuretic effect

The aqueous extract of coriander increased dieresis and the urinary excretion of sodium, potassium, chloride and the glomerular filtration rate at doses of 40 and 100 mg/kg administered by intravenous infusion (120 min) in anesthetised Wistar rats. The mechanism of diuretic action of coriander appeared to be similar to that of furosemide.[18] Rodents treated with *C.sativum* crude extract (Cs.Cr), a mild increase in the urine out put was observed at the dose of 30 mg/kg (5.1 ± 0.60 ml), while a significant diuretic effect ($p < 0.01$) was caused by the dose of 100 mg/kg (6.47 ± 0.44 ml). An increase in the urine volume was evident within 1 h of administration of furosemide, while the onset of diuretic effect was 3-4 hr with Cs.Cr.[45]

Anti-fungal activity

Coriander oil did not have an effect on mycelia growth (*A. Parasiticus*) and did not affect the aflatoxin content of the fungus.[46] This antimicrobial activity against bacteria and fungi has also been demonstrated in essential oils extracted from C. sativum seed.[44]

Other studies and effects

A polyherbal Ayurvedic formulation containing ripe fruits of coriander as one of its major ingredient was tested on two different experimental animal models of inflammatory bowel disease (acetic acid induced colitis in mice and indomethacin induced enterocolitis in rats). Obtained results shown that the formulation was efficacious against inflammatory bowel disease.[47] Coriander also suggested having preventive effect on localised lead decomposition in ICR mice. The administration of *C. sativum* significantly decreased lead decomposition in the femur and reduced the severe lead-induced injury in the kidney of ICR mice which were given lead (1000 ppm) as lead acetate trihydrate in drinking water for 32 days.[48] The effect of the aqueous extract of fresh coriander seeds on female fertility in rats was studied. The extract (250 and 500 mg/kg orally) produced a dose-dependent significant anti-implantation effect, but failed to produce complete infertility.[49]

REFERENCES

1. Weiss, E.A. *Spice Crops*. CAB International, Wallingford, UK. 2002; 411.
2. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. Food Chemistry. 2006; 88:293-297.
3. Baba K, Xiao YQ, Taniguchi M, Ohishi H, Kozawa M. Isocoumarins from *Coriandrum sativum*. Phytochemistry. 1991; 30(12):4143-4146.
4. Msaada K, Hosni K, Taarit MB, Chahed T, Kchouk ME, Marzouk B. Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. Food Chemistry. 2007; 102:1131-1134.
5. Aluko RE, McIntosh T, Reaney M. Comparative study of the emulsifying and foaming properties of defatted coriander (*Coriandrum sativum*) seed flour and protein concentrate. Food Research International. 2001; 34:733-738.
6. Eyres G, Marriott PJ, Dufour JP. The combination of gas chromatography-olfactometry and multidimensional gas chromatography for the characterisation of essential oils. Journal of Chromatography A. 2007; 1150:70-77.
7. Lawrence BM. A planning scheme to evaluate new aromatic plants for the flavor and fragrance industries. In: New crops. Janick J, Simon JE (eds). New York, Wiley, p. 620-27; 1993.
8. Coskuner Y, Karababa E. Physical properties of coriander seeds (*Coriandrum sativum* L.). Journal of Food Engineering. 2006; 80:408-416.
9. Guenther E. The essential oil. Vol. IV. Florida, USA, REK Publishing Company, p. 602-15; 1950.
10. Eikani MH, Golmohammad F, Rowshanzamir S. Subcritical water extraction of essential oils from coriander seeds (*Coriandrum sativum* L.). Journal of Food Engineering. 2007; 80:735-740.
11. Rastogi RP, Mehrotra BN. Compendium of Indian medicinal Plants. Vol. II. Lucknow, CDRI, p. 212; 1993.
12. Telci I, Gul TO, Sahbaz N. Yield, essential oil content and composition of Coriandrum sativum varieties (var. Vulgare Alef and var. microcarpum DC.) grown in two different locations. J Essentl Oil Res. 2006; 18:189-93.
13. Millam S, Mitchell S, Craig A, Paoli M, Moscheni E, Angelini L. *In vitro* manipulation as a means for accelerated improvement of some new potential oil crop species. Industrial Crops and Products. 1997; 6:213-219.
14. Kim SW, Park MK, Bae KS, Rhee MS, Liu JR. Production of petroselenic acid from cell suspension cultures of *Coriandrum sativum*. Phytochemistry. 1996; 42(6):1581-1582.
15. Ozcan MM, Unver A, Uçar T, Arslan D. Mineral content of some herbs and herbal teas by infusion and decoction. Food Chemistry. 2008; 106:1120-1127.
16. Al-Bataina BA, Maslat AO, Al-Kofahi MM. Element analysis and biological studies on ten oriental spices using XRF and Ames test. J. Trace Elem. Med Biol. 2003; 17(2):85-90.
17. Matthaus B, Angelini LG. Anti-nutritive constituents in oilseed crop from Italy. Industrial Crops and Products. 2005; 21:89-99.
18. Aissaoui A, El-Hilaly J, Israili ZH, Lyoussi B. Acute diuretic effect of continuous intravenous infusion of an aqueous extract of *Coriandrum sativum* L. in anesthetized rats. J of Ethnopharmacol. 2008; 115:89-95.
19. Emamghoreishi M, Khasaki Aazam MF. *Coriandrum sativum*: evaluation of its anxiolytic effect in the elevated plus-maze. J of Ethnopharmacol. 2005; 96:365-370.
20. Anonymous. The wealth of India: Raw materials. Vol. II, CSIR, New Delhi, India, p. 347-50; 1950.
21. Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian medicinal plants with active principles. Part I. New Delhi, India, P & I Directorate, CSIR. p. 232-33; 1992.
22. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi, India, CSIR,. p. 77-78; 1956.
23. Yusuf M, Chowdhuty JU, Wahab MA, Begum J. Medicinal plants of Bangladesh. Bangladesh, Bangladesh Council of Scientific and Industrial Research, p. 66; 1994.
24. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. 2nd ed. Dhaka, Asiatic Society of Bangladesh, p. 183; 2003.
25. Chitra V, Leelamma S. *Coriandrum sativum* – mechanism of hypoglycemic action. Food Chemistry. 1999; 67:229-231.
26. Gallagher AM, Flatt PR, Duffy G, Abdel-Wahab YHA. The effects of traditional antidiabetic plants on *in vitro* glucose diffusion. Nutrition Research. 2009; 23:413-424.

27. Al-Mofleh IA, Alhaider AA, Mossa JS, Al-Sohaibani MO, Rafatullah S, Qureshi S. Protection of gastric mucosal damage by *Coriandrum sativum* L. Pretreatment in Wistar albino rats. Environmental Toxicology and Pharmacology. 2006; 22:64-69.
28. Gray AM, Flatt PR. Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant Coriandrum sativum (coriander). Br J Nutr. 1999; 81:203-9.
29. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes: studies in normal and streptozotocin diabetic mice. Diabetologia. 1990; 33:462-4
30. Shyamala BN, Gupta S, Lakshmi AJ, Prakash J. Leafy vegetable extracts – antioxidant activity and effect on storage stability of heated oils. Innovative Food Science and Emerging Technologies. 2005; 6:239-245.
31. Sujatha R, Srinivas L. Modulation of lipid peroxidation by dietary components. Toxic *in vitro*. 1995; 9(3):231-236.
32. Yepez B, Espinosa M, Lopez S, Bolanos G. Producing antioxidant fractions from herbacious matrices by supercritical fluid extraction. Fluid Phase Equilibria. 2002; 184-197:879-884.
33. Wong PYY, Kitts DD. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselenium crispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chemistry. 2006; 97:505-515.
34. Melo EA, Filho JM, Guerra NB. Characterization of antioxidant compounds in aqueous coriander extract (*Coriandrum sativum* L.). Lebensm.-Wiss. u.-Technol. 2005; 38:15-19.
35. Wangensteen H, Samuelsen AB, Malterud KE. Antioxidant activity in extracts from coriander. Food Chemistry. 2004; 88:293-297.
36. Anilakumar KR, Nagaraj NS, Santhanam K. Effect of coriander seeds on hexachlorocyclohexane induced lipid peroxidation in rat liver. Nutrition Research. 2001; 21:1455-1462.
37. Chitra V, Leelamma. *Coriandrum sativum* – effect on lipid metabolism in 1,2-dimethyl hydrazine induced colon cancer. J of Ethnopharmacol. 2000; 71:457-463.
38. Cortes-Eslava J, Gomez-Arroyo S, Villalobos-Pietrini R, Espinosa-Aguirre JJ. Antimutagenicity of coriander (Coriandrum sativum) juice on the mutagenesis produced by plant metabolites of aromatic amines. Toxicology Letters. 2004; 153:283-292.
39. Bakkali F, Averbeck S, Averbeck D, Zhiri A, Idaomar M. Cytotoxicity and gene induction by some essential oils in the yeast *Saccharomyces cerevisiae*. Mutation Research. 2005; 585:1-13.
40. Cherng JM, Chiang W, Chiang LC. Immunomodulatory activities of common vegetables and spices of Umbel liferae and its related coumarins and flavonoids. Food Chemistry. 2008; 106:944-950.
41. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. *In vitro* and *in vivo* anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J of Ethnopharmacol. 2007; 110:428-433.
42. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. International Journal of Food Microbiology. 2002; 74: 101-109.
43. Begnami AF, Duarte MCT, Furletti V, Rehder VLG. Antimicrobial potential of Coriandrum sativum L. against different Candida species *in vitro*. Food Chemistry. 2010; 118:74-77.
44. Cantore L, Iacobellis P, Marco DP, Capasso A, & Senatore F. (2004). Antibacterial activity of Coriandrum sativum L. and Foeniculum vulgare miller var. vulgare (miller) essential oils. Journal of Agricultural and Food Chemistry. 2004; 52:7862-786.
45. Jabeen Q, Bashir S, Lyoussi B, Gilani AH. Coriander fruit exhibits gut modulatory, blood pressure lowering and diuretic activities. Journal of Ethnopharmacology. 2009; 122:123-130.
46. Atanda OO, Akpan I, Oluwafemi F. The potential of some spice essential oils in the control of *A. Parasiticus* CFR 223 and aflatoxin production. Food Control. 2007; 18:601-607.
47. Jagtap AG, Shirke SS, Phadke AS. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. J Ethnopharmacol. 2004; 90:195-204.
48. Aga M, Iwaki K, Ueda Y, Ushio S, Masaki N, Fukuda S et al,. Preventive effect of *Coriandrum sativum* (Chinese parsley) on localized lead deposition in ICR mice. J of Ethnopharmacol. 2001; 77:203-208.
49. Al-Said MS, Al-Khamis KI, Islam MW, Parmar NS, Tariq M, Ageel AM. Post-coital antifertility activity of the seeds of *Coriandrum sativum* in rats. J of Ethnopharmacol. 1987; 21:165-173.

Monograph of *Psidium guajava* L. leaves

A. M. Metwally, A. A. Omar, N. M. Ghazy and F. M. Harraz, S. M. El Sohafy*

S. M. El Sohafy, El Khartoum Square, Azarita, Faculty of pharmacy, Alexandria, Egypt.

ABSTRACT

The following article is a detailed monograph of *Psidium guajava* L. leaves containing all description of the leaves concerning its botany, chemistry and activity.

Key words: *Psidium guajava* L. Leaf, Guava leaf, Guava monograph

INTRODUCTION

Guava leaf is a main ingredient in many herbal mixtures marketed in the Egyptian market and worldwide. It has been stated very often that the lack of standardization of herbal remedies and plant medicines is holding back the use of medicinal plants in the modern system of medicine, therefore a growing demand for the establishment of a system of standardization for every herbal preparation in the market is required and the buildup of a monograph containing all information required about the herbal drug is necessary.

MATERIALS AND METHODS

Plant materials

Psidium guajava L. leaf used for the phytochemical investigation and the antimicrobial studies was collected from El tahrir (Alexanria – Cairo road at kilo 47). Samples of *Psidium guajava* L. leaf used for the comparative studies were collected from different localities in Egypt as indicated in the appendices below.

Reference materials

Quercetin, glucose, galactose, L-arabinose and D-arabinose were supplied by E. Merck (Darmstadt, Germany). Quercetin-3-β-D-glucoside and quercetin-3-β-D-galactoside were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Address for correspondence:
E-mail: Samahelsohafy@yahoo.com

DOI: 10.5530/pj.2011.21.17

Solvents

The solvents used in this work; petroleum ether (40-60°C), ether, chloroform, ethyl acetate, butanol, methanol and ethanol were of analytical grade.

Chromatographic requirements

Precoated HPTLC plates (silica gel 60F-254) with adsorbent layer thickness 0.2 mm, E- Merck, Darmstadt, Germany.

HPTLC equipments

Sample solutions were applied by means of a Camag (Wilmington, NC) Linomat IV automated spray-on band applicator equipped with a 100-µl syringe. Zones were quantified by linear scanning at 254 nm with a Camag TLC Scanner 3 with a deuterium source in the reflection mode. The peak areas of the chromatograms' spots were determined using CATS TLC software and winCATS TLC software (version 4.X).

NOMENCLATURE

Botanical Nomenclature

Psidium guajava L.

Botanical Family

Myrtaceae

Definition

Guava consists of the dried leaves of *Psidium guajava* L. family *Myrtaceae*.

Common Names

Guava (Egypt, USA, latin America, Asia, Africa), guyaba (Cuba), guayaba (Guatemala, Nicaragua, Paraguay), amrood (India).[1-6]

HISTORY

Guava is native to the American tropics. The English name guava probably came from the Haitian name, guajaba. The Spanish explorers took the guava to the Philippines and the Portuguese disseminated it from the Philippines to India. Then it spreaded easily and rapidly throughout the tropics because of the abundance of seeds with long viability and became naturalized to the extent that people in different countries considered the guava to be indigenous to their own region. It is now also grown in the subtropics.[2]

IDENTIFICATION

Botanical Identification

Macroscopic Identification (Figures 1, 2)

Habit: Medium sized tree with thin smooth, patchy, peeling whitish brown bark,[1,5] but under high moisture conditions, grows to 6-9 m in height.[2]

Root: Tap, branched.[1]

Stem: Erect, aerial, woody, branched, cylindrical, solid, glabrous, white or brown.[1]

Leaves: Simple, alternate, short-petiolate, exstipulate, gland dotted, aromatic, entire, apex ovate.[1]

The leaves are 10-12 cm in length, 5-7 cm in width. They have a green colour and leathery texture.

The lamina is green, simple with acute apex, entire margin, symmetric – asymmetric base. The vennation is pinnate reticulate. The midrib is more prominent on the lower surface. The upper surface is slightly paler in colour than the lower surface. Both surfaces are pubescent.

The petiole is short (0.3-0.4 in length and 0.2-0.3 in diameter), green in colour, showing a groove on the upper surface and hairy.

Inflorescence: Cymose, solitary and axillary.[1]

Flower: Flowers occur singly or in clusters of 2 to 3 at the leaf axils of current and preceding growth.[5] Flowers are Pedicellate, bracteate, complete, hermaphrodite, actinomorphic, epigynous, pentamerous, cyclic, white.[1]

Calyx: 4-5 sepals,[3,5]gamosepalous, reduced, fused.[1]

Corolla: 4-5 petals,[3,5]gamopetalous, forming a corolla cap covered by calyx cap, the so formed calyptra falls off when flower opens, superior.[1]

Androecium: Stamens indefinite, polyandrous, attached on the rim of calyx cap, folded inwards in bud condition,

Figure 1: Photography of *Psidium guajava* L. leaf.

Figure 2: *Psidium guajava* L. leaf (x1).

anthers dorsifixed, versatile, bicelled, small, dehisce longitudinally.[1]

Gynoecium: 4-5 carpels, style, stigma minute, quadra- penta-locular, [3,5] syncarpous, ovary inferior, axile placentation.[1]

Fruit: The fruit is a many-seeded berry, varying in size from 2.5 to 10 cm in diameter. The shape can be globose, ovoid, elongated or pear-shaped. Skin colour is yellow when ripe but flesh colour may be pink, salmon, white or yellow. Skin texture may be smooth or rough. The inner wall of the carpels is fleshy and of varying thickness and seeds are embedded in the pulp. Flavour and aroma vary widely among seedling populations.[2]

Microscopic Identification (Figures 3, 4, 5, 6, 7)

1. The leaf lamina

A transverse section in the leaf (Figures 3, 4, 5) shows upper and lower epidermises, hypodermis and a dorsiventral mesophyll. The palisade tissue consists of two rows of columnar cells and is discontinuous in the midrib region. The midrib is more prominent in the lower side and shows bicollateral arc-shaped vascular bundle which is surrounded by two arcs of pericyclic fibers above and below the bundle.

The Epidermises: The upper epidermis of the lamina (Figure 6 *u. ep.*) consists of polygonal, nearly isodiametric or slightly elongated cells with straight, slightly thick anticlinal walls covered with thin smooth cuticle and devoid of stomata. The lower epidermis (Figure 6 *l. ep.*) consists of polygonal, nearly isodiametric cells with straight walls and covered with thin, smooth cuticle. Stomata are present on the lower epidermis only. They are oval in shape and of the paracytic type.

Trichomes (Figure 6 *t.*) are present in both epidermises, being more numerous in the upper epidermis. They are non-glandular unicellular wooly straight, curved or twisted, covered with thick smooth cuticle, arising from a cicatrix surrounded by radiating epidermal cells. Measurements of upper and lower epidermisesare shown in table 1.

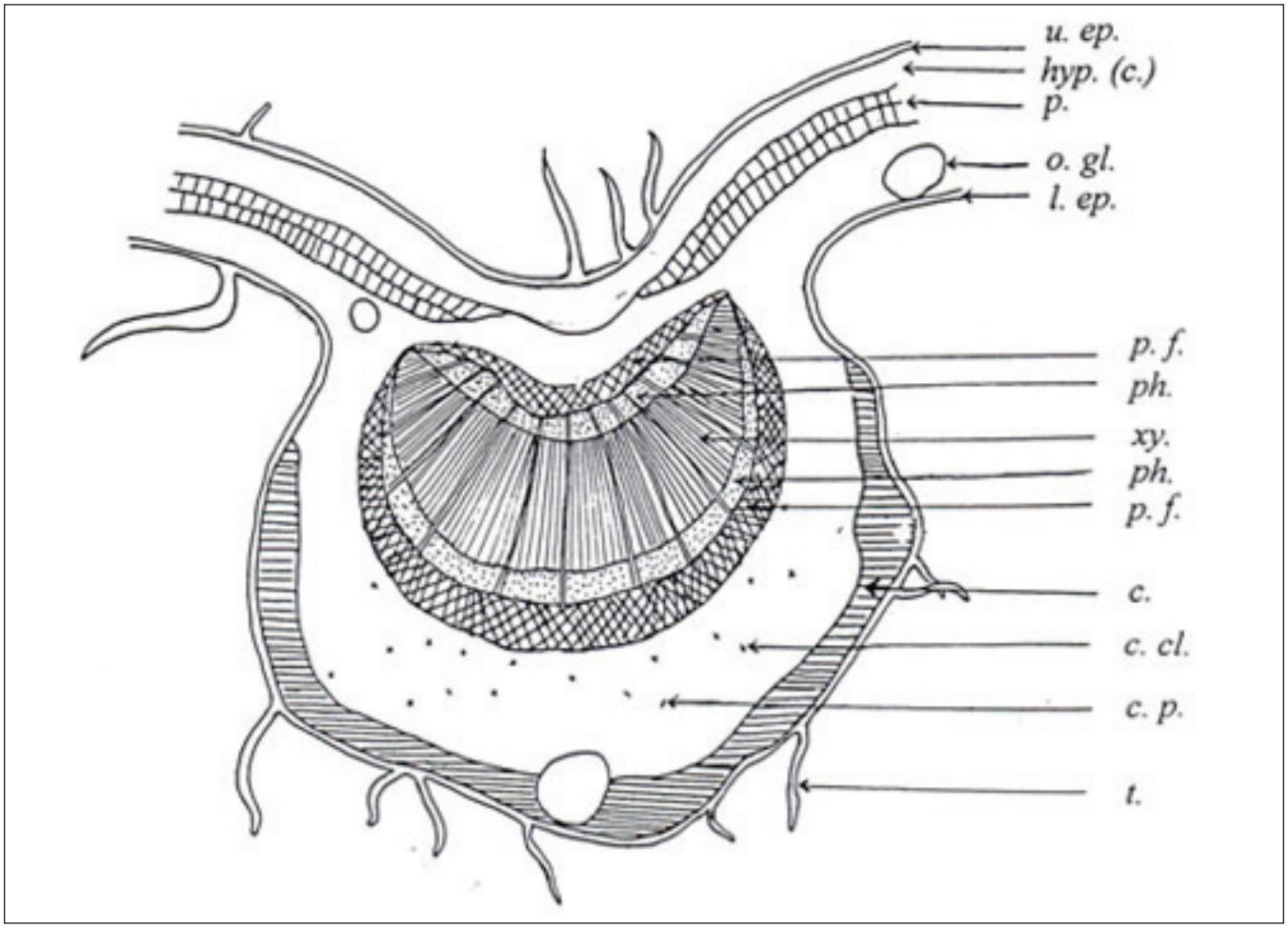


Figure 3: Diagram of a transverse section of *Psidium guajava* L. leaf (x).
c. *collenchyma;* c. cl. *calcium oxalate cluster crystal;* c. p. *calcium oxalate prism;* hyp. *hypodermis;* l. ep. *lower epidermis;* o. gl. *oil gland;* p. *palisade;* p. f. *pericyclic fibres;* ph. *phloem;* t. *trichome;* u. ep. *upper epidermis;* xy. *xylem.*

The hypodermis : The hypodermis consists of 2-3 layers of collenchymatous cells.

The mesophyll: It is dorsiventral, the palisade is discontinuous over the midrib region and is formed of 2-3 rows of columnar cells, having straight anticlinal walls and containing green plastids. The spongy tissue is formed of 5-8 rows of more or less spherical cells and containing green plastids. Small vascular bundles and shizogenous and schizolysigenous oil glands may be embedded within the spongy tissue.

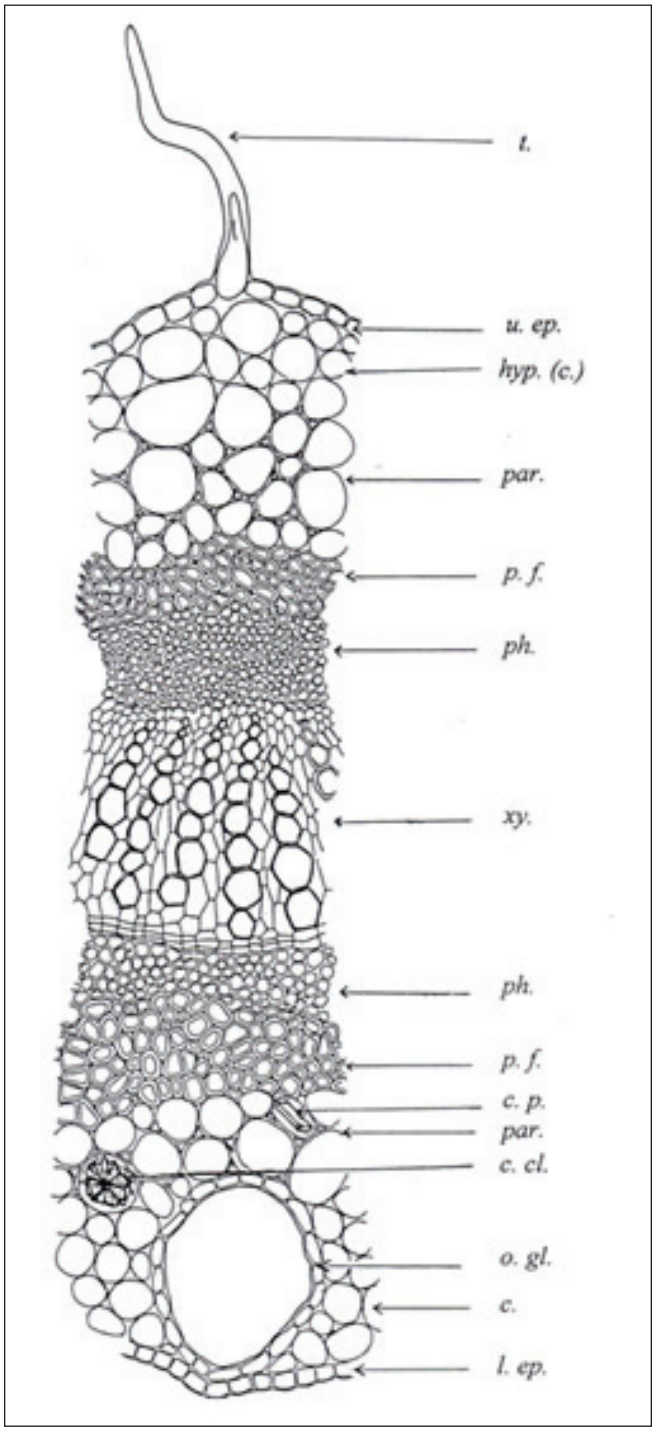


Figure 4: Detailed transverse section of the midrib of *Psidium guajava* L. leaf (x).
c. *collenchyma;* c. cl. *calcium oxalate cluster crystal;* c. p. *calcium oxalate prism;* hyp. *hypodermis;* l. ep. *lower epidermis;* o. gl. *oil gland;* p. *palisade;* p. f. *pericyclic fibres;* ph. *phloem;* t. *trichome;* u. ep. *upper epidermis;* xy. *xylem.*

The midrib: The cortical tissue of the midrib consists of 2-3 rows of collenchymatous cells beneath the upper epidermis and 3-4 rows abutting the lower epidermis followed by 3-4 layers of thin walled parenchyma with distinct intercellular spaces. The parenchyma cells contain few prisms and numerous clusters of calcium oxalate. Shizosenous and schizolysigenous oil glands are also present. The pericycle is formed of two arcs of lignified fibres above and below the vascular bundle. The fibres (Figure 6 *f.*) are long (80.45-91.95 μm in length and 6.89-17.24 μm in width), fusiform with wavy lignified walls, rather wide lumen and more or less acute apices.

The vascular tissue: It consists of an arc-shaped bicollateral vascular tissue which is formed of xylem and two arcs of phloem above and below it. The xylem consists of lignified spiral, annular vessels and wood parenchyma. The phloem consists of sieve elements and phloem parenchyma.

2. The leaf petiole

A transverse section in the petiole (Figure 7) is planoconvex, slightly grooved on the upper part. It is formed of an epidermis followed by the cortex which is formed of parenchymatous tissue containing prisms

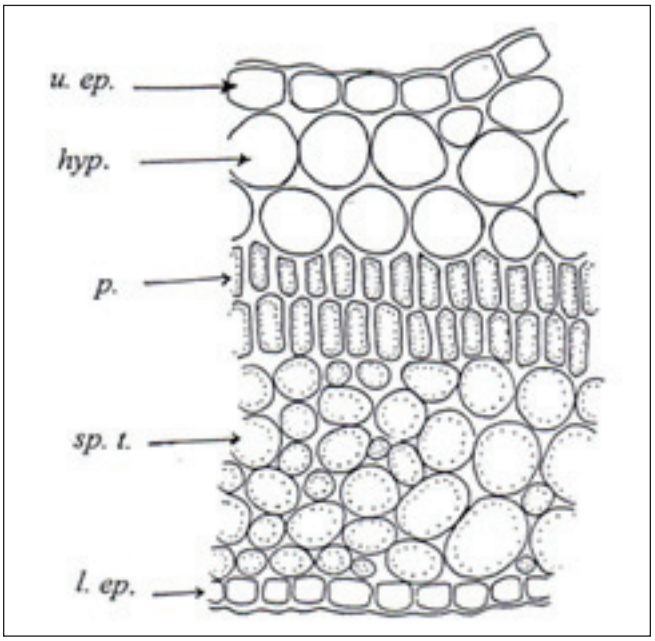


Figure 5: Detailed transverse section of the lamina of *Psidium guajava* L. leaf (x).
hyp. *hypodermis;* l. ep. *lower epidermis;* sp. t. *spongy tissue;* u. ep. *upper epidermis.*

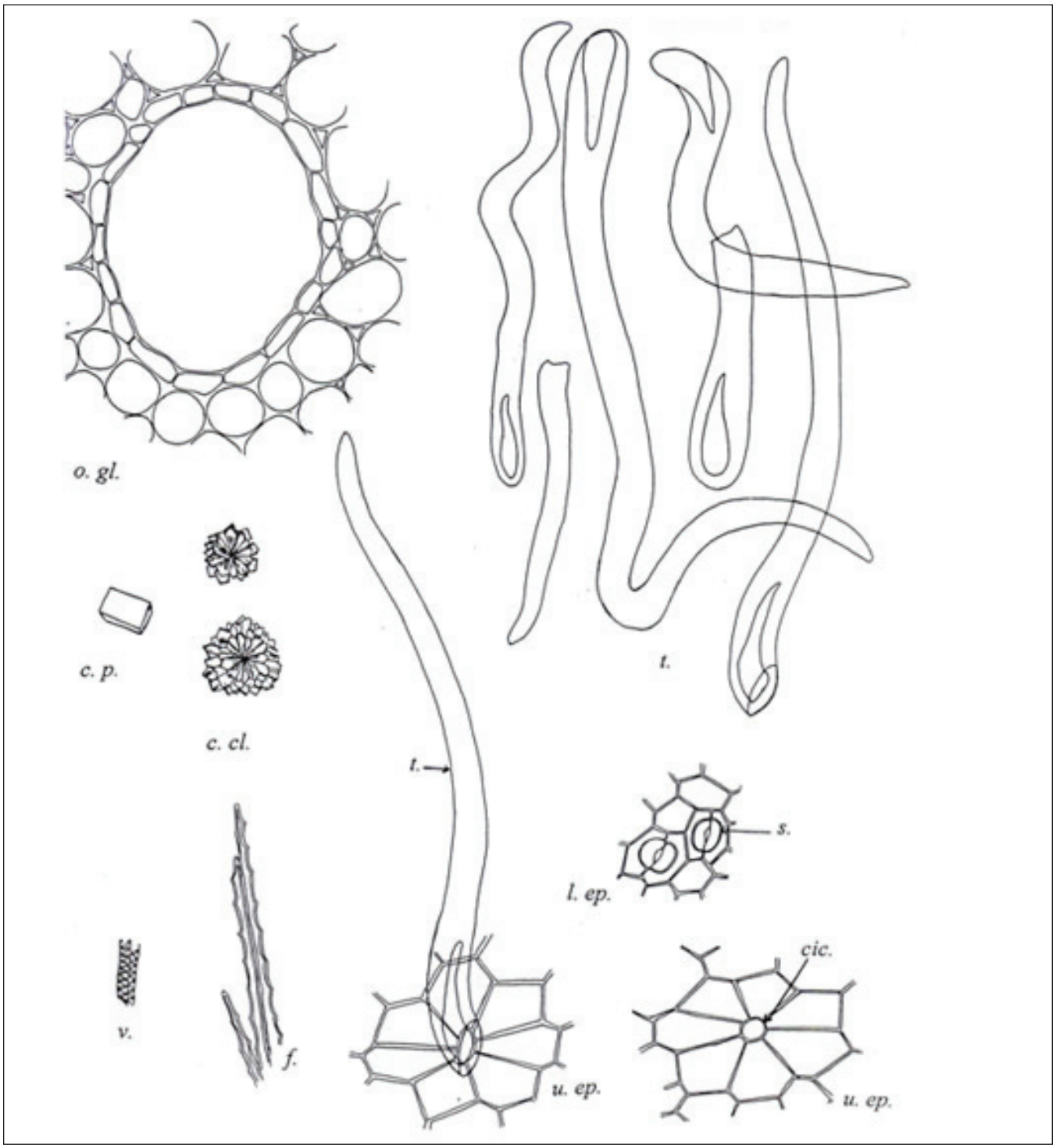


Figure 6: Elements of *Psidium guajava* L. leaf (x).
cic. *cicatrix;* c. cl. *calcium oxalate cluster crystal;* c. p. *calcium oxalate prism;* f. *fibre;* l. ep. *lower epidermis;* o. gl. *oil gland;* s. *stomata;* t. *trichome;* u. ep. *upper epidermis;* v. *vessel.*

and clusters of calcium oxalate and one row of shizogenous and shizolysigenous oil glands situated in the outer region of the cortex. The cortex is traversed by crescent shaped vascular tissue similar to that present in the leaf.

3. Powdered leaf (Figure 6)

Characteristic Features The dried powdered leaf is light green in colour with an aromatic taste and a characteristic odour. It is characterized microscopically by the following elements:

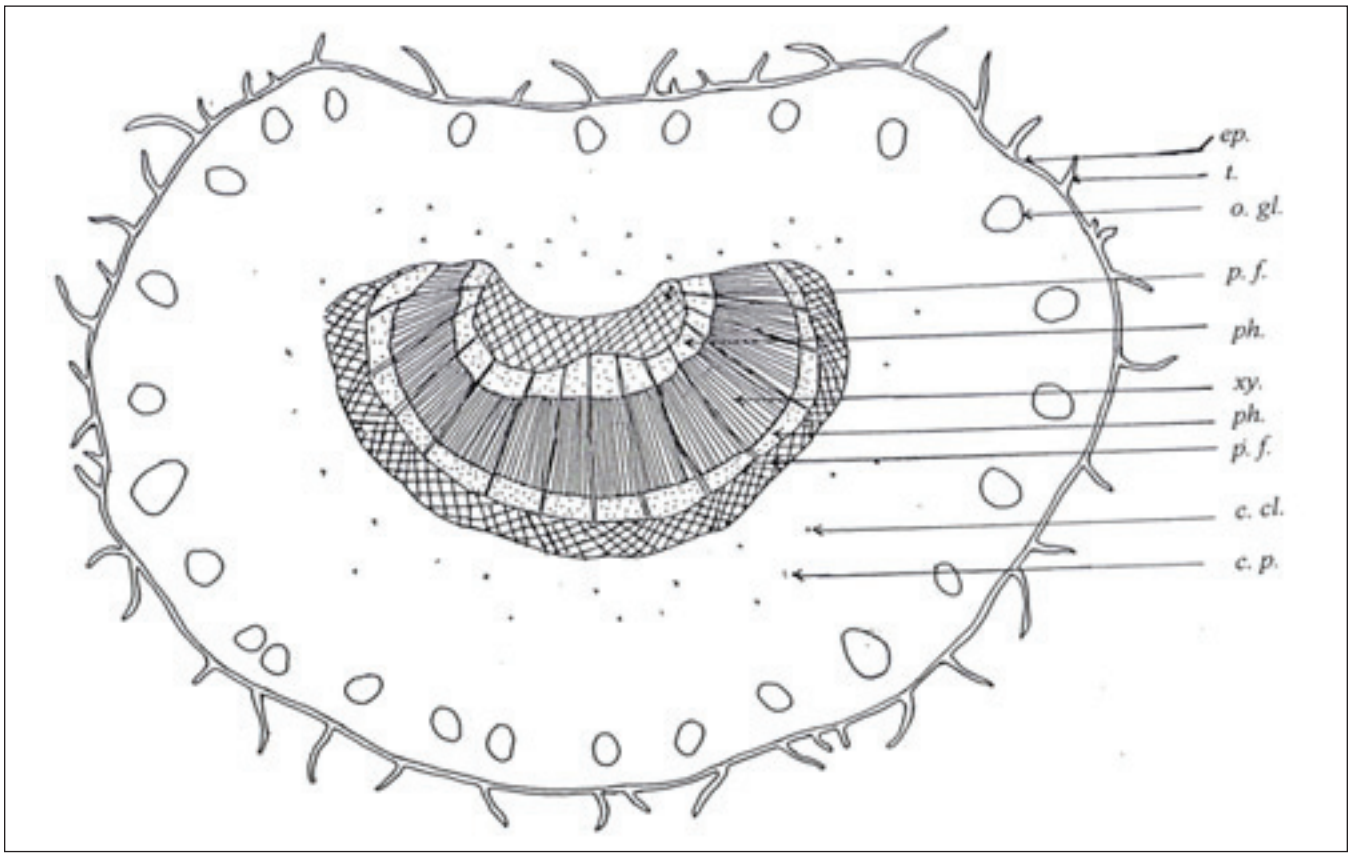


Figure 7: Diagram of a transverse section of *Psidium guajava* L. leaf petiole(x).
c. cl. *calcium oxalate cluster crystal;* c. p. *calcium oxalate prism;* ep. *epidermis;* o. gl. *oil gland;* p. f. *pericyclic fibers;* ph. *phloem;* t. *trichome;* xy. *xylem.*

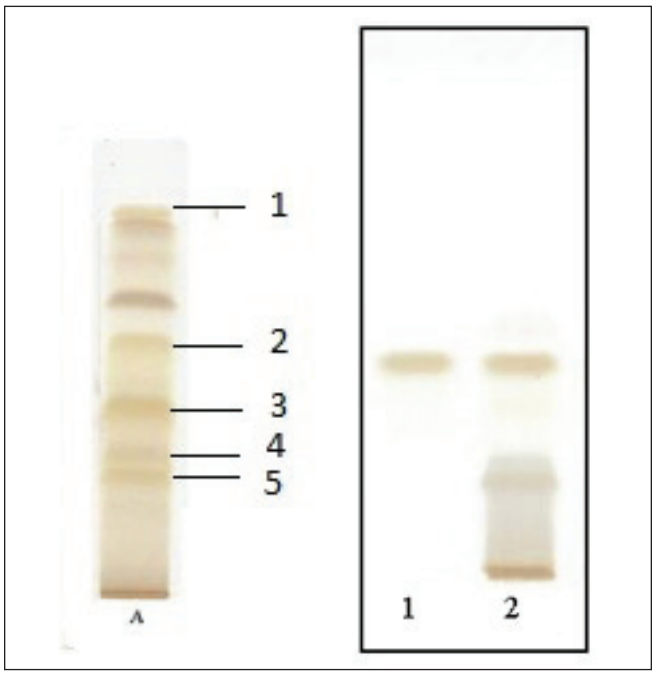


Figure 8: Typical ethyl acetate pattern of guava leaf.
A: Ethyl acetate extracte of guava leaf, 1: Quercetin, 2: Quercetin-3-O-α-L-arabinofuranoside, 3: Quercetin-3-O-β-D-arabinopyranoside, 4: Quercetin-3-glucoside and 5: Quercetin-3-galactoside.

Table 1: Measurements of upper and lower epidermises.

	Length (µm)	Width (µm)
Upper epidermis	17.2 - ***23*** - 28.7	16.1 - ***17.2*** - 18.4
Cells of cicatrix	13.8 - ***25.3*** - 34.5	11.5 - ***17.2*** - 23
Lower epidermis	15.3 - ***16.1*** - 17.2	9.8 - ***10.3*** - 10.6
Stomata	11.5 - ***12*** - 12.6	9 - ***9.8*** - 10.3
Subsidiary cells	19.5 - ***20.7*** - 21.8	10.3 - ***11.5*** - 12.6

1. Numerous unicellular wooly large characteristic nonglandular trichomes, strongly thickened with smooth cuticle.
2. Numerous fragments of broken nonglandular trichomes.
3. Fragments of upper epidermis which consists of polygonal isodiametric cells with straight walls, covered with smooth cuticle showing cicatrix and devoid of stomata. Few cells bear the previously described trichomes.
4. Fragments of lower epidermis which consists of polygonal isodiametric cells with straight walls, covered with smooth cuticle showing paracytic stomata and bearing numerous trichomes.
5. Fragments of the leaf lamina in sectional view.

6. Fragments of cortical parenchyma showing shizogenous and shizolysigenous oil glands, numerous clusters and few prisms of calcium oxalate.
7. Numerous clusters and few prisms of calcium oxalate.
8. Fragments of lignified pericyclic fibres with wavy walls and acute apices.
9. Fragments of lignified spiral and annular vessels.

Identification by TLC

Extract 2 g powdered Guava leaves with 20.0 ml of boiling water for 10 minutes, cool and filter. Concentrate 10 ml of this filterate to about 5 ml then shake with 10 ml of ethyl acetate. Separate the ethyl acetate layer, dry and redissolve in 2 ml of methanol. Examine by TLC using the references mentioned below. For TLC examination use silica gel G layers, 0.25 mm thick and ethyl acetate - methanol - water -acetic acid (100:2:1:4 drops) as developing solvent and ammonia as revealing reagent. Detect the resolved bands by UV. The Following figure is the typical ethyl acetate pattern.

CONSTITUENTS

1. Flavonoids

i. Quercetin and its glycosides:
A summary of the isolated quercetin and itsglycosides are given in table 2.

ii. Other flavonoids include morin-3-O-α-L-lyxopyranoside,[10] morin-3-O-α-L-arabinopyranoside,[10] kæmpferol,[7] luteolin-7-O-glucoside[7] and apigenin-7-O-glucoside.[7]

2. Tannins

i. Amritoside (ellagic acid 4-gentiobioside).[11]

ii. Guavin B(structure I)[14] and Guavins A, C and D (structuresΠ, III, IV respectively).[15]

iii. Antidiabetic agents: Isostrictinin (V),[16] Strictinin (VI),[16] Pedunculagin (VII).[16]

iv. Antimutagenic agents: (+)-gallocatechin (a bio-antimutagenic compound against UV induced mutation in Escherichia coli).[17]

Structures of the isolated tannins are given in table 3.

3. Isoprenoids

i. Monoterpenes:
Caryophyllene oxide,[18,19] β-selinene,[18] 1,8-cineole, α-pinene,[19,20] myrcene , δ-elemene, d-limonene, caryophyllene,[7,19] linalool,[19] eugenol, β-bisabolol,[20] β-bisabolene, β-sesquiphellandrene,[21] Me 2-methyl-thiazolidine-4-(R)-carboxylate (cis and trans), ethyl 2-methyl-thiazolidine-4-(R)-carboxylate (cis and trans),[22] aromadendrene, α- and β-selinene, caryophellene epoxide, cayophylladienol,[23] (E)-nerolidol, Selin-11-en-4-alpha-ol.[24]

ii. Terpenoids:
A summary of the isolated triterpenoidal compounds are given in table 4.

Table 2: Quercetin and its glycosides of guava leaves.

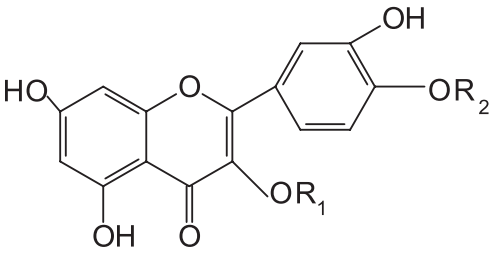
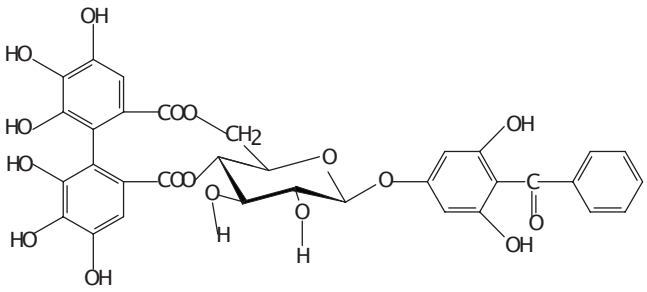
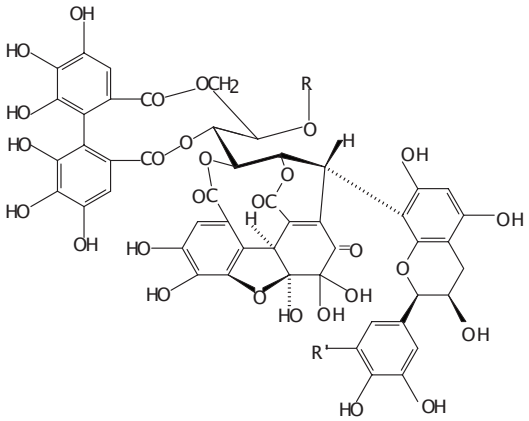
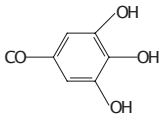
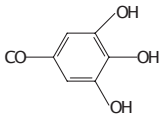
Flavonoid	R_1	R_2	References
			
Quercetin	H	H	7, 8, 9, 10, 11
Avicularin: Quercetin 3-O-L-arabinofuranoside	L-arabinofuranose	H	9
Guaijaverin: Quercetin 3-O-α-L-arabinopyranoside	α-L-arabinopyranose	H	8, 9, 10, 11
Isoquercetin: Quercetin 3-O-β-D-glucoside.	β-D-glucose	H	8, 12
Hyperin: Quercetin 3-O-β-D-galactoside.	β-D-galactose	H	8
Quercitrin: Quercetin 3-O-β-L-rhamnoside.	β-L-rhamnose	H	7, 8, 12
Quercetin 3-O-β-D arabinopyranoside	β-D-arabinopyranose	H	13
Quercetin 3-O-gentiobioside	Gentiobiose	H	8
Quercetin 4'-glucuronoide	H	Glucuronic acid	12

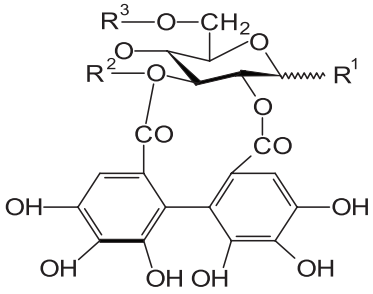
Table 3: Tannins isolated from guava leaves.



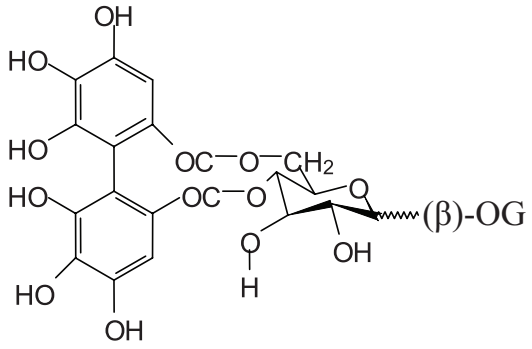
I



II	III	IV
R			$COCH_3$
R'	H	OH	H



(V): R^1 = (β)-OG, R^2 = R^3 = H
(VII): R^1 = OH, R^2 ~ R^3 = (S)-HHDP (hexahydroxydiphenoyl)



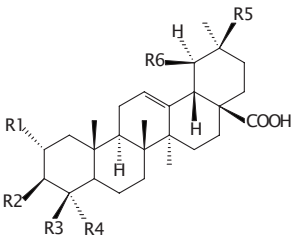
VI

PHARMACOLOGICAL ACTIONS OF *P. GUAJAVA* L. LEAF

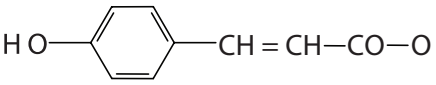
1. Anticough action

Guava leaf has been used in Bolivia and Egypt for a long time to treat ailments including cough and pulmonary diseases.[29] The aqueous extract decreased the frequency of cough induced by capsaicin aerosol within 10 minutes after intraperitoneal injection of the extract. The LD_{50} of guava leaf extract was more than 5 g/kg. These results suggested that guava leaf extract is recommended as a cough remedy.[30] Meanwhile a recent study conducted on the Egyptian plant showed that the alcoholic extract (in a dose starting from 4 μg/ml), the aqueous extract(from 8 μg/ml), the ethyl acetate extract (from 6 μg/ml), the essential oil (16 μg/ml) as well as quercetin (30 μg/ml) produces a significant drop in contractile

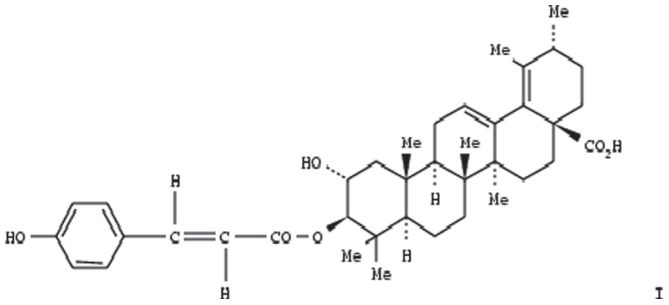
Table 4: Terpenoids previously isolated from guava leaves.



R

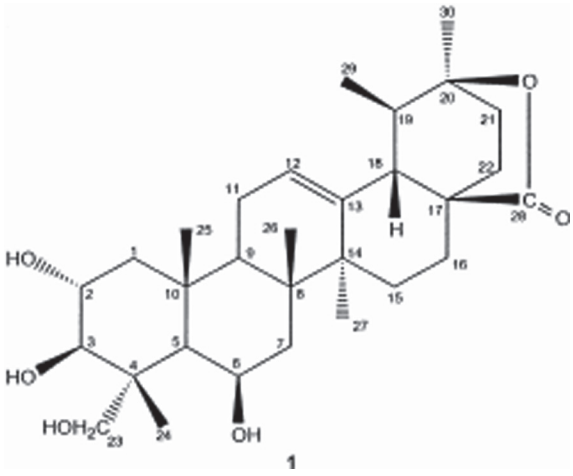


	R1	R2	R3	R4	R5	R6	Ref.
Guavanoic acid	OH	OH	CH_3-	CH_3-	-OAc	CH_3-	25
Guavacoumaric acid	OH	OH	R	CH_3-	H	CH_3-	25
Guajanoic acid	OMe	R	CH_3-	CH_3-	H	CH_3-	26
Ursolic acid	H	OH	CH_3-	CH_3-	H	CH_3-	26
2α-hydroxyursolic acid	OH	OH	CH_3-	CH_3-	H	CH_3-	25
Maslinic acid	OH	OH	CH_3-	CH_3-	CH_3-	H	27
Asiatic acid	OH	OH	CH_3-	CH_2OH	H	CH_3-	25
Jacoumaric acid	OH	R	CH_3-	CH_3-	H	CH_3-	25
Isoneriucoumaric acid	R	OH	CH_3-	CH_3-	H	CH_3-	25
Guajavanoic acid							26



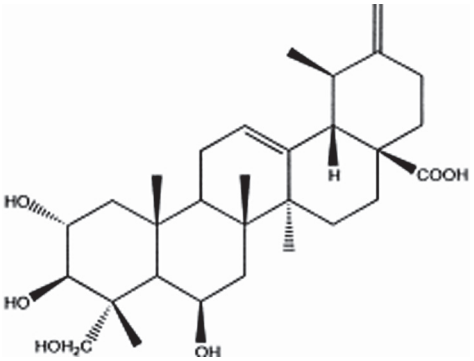
I

Guajavolide							28



1

Guavenoic acid							28



response of isolated guinea pig trachea treated with histamine (2 μg/ml), acetyl choline (1 μg/ml) or serotonine (1 μg/ml). This study concluded that the extracts as well as the essential oil are safe for use as anti-cough concerning their effect on isolated trachea, their smooth muscle relaxant and their anti-inflammatory effects. Large doses may inhibit the ventricular contraction of the heart as tested on isolated rabbit heart.[7]

Moreover the high percentage of essential oil (0.46%) and its broad antimicrobial activity [23] may be beneficial in cough treatment.[7]

2. Spasmolytic activity

Several researches assure that guava leaf extracts possess a spasmolytic activity[7,8,31-36] which is mainly attributed to the polyphenolic fraction[36] and is due to the aglycone quercetin (guava leaf is a rich source of quercetin glycosides which are hydrolyzed by the gastrointestinal fluid to give the aglycone quercetin).[8] Quercetin produces smooth muscle relaxation on isolated guinea pig ileum previously contracted by a depolarizing KCl solution and also inhibits intestinal contraction induced by different concentrations of Ca^{2+}.[31] Furthermore, the study conducted on the Egyptian plant showed that the alcoholic extract (4 μg/ml), the aqueous extract (8 μg/ml), the ethyl acetate extract (6 μg/ml), the essential oil (16 μg/ml) as well as quercetin (30 μg/ml) produced a significant muscle relaxant effect on isolated guinea pig ileum and rabbit intestine previously treated with histamine (2 μg/ml).[7]

Asiatic acid isolated from guava leaf also showed dose-dependant (10-500 μg/ml) spasmolytic activity in spontaneously contracting isolated rabbit jejunum preparations.[25]

3. Antibacterial activity

A summary of the antibacterial studies previously done for the leaves and its fractions are given in table 5. Flavonoids morin-3-O-alpha-L-lyxopyranoside and morin-3-O-alpha-L-arabopyranoside were recently isolated from the leaves and have MIC of 200 μg/ml for *Salmonella enteritidis*, and 250 μg/ml and 300 μg/ml against *Bacillus cereus*, respectively.[10]

4. Antiamoebic activity

The leaf possesses antiamoebic activity which is concentrated in the polyphenolic fraction.[36]

5. Antifungal activity

P. guajava L. leaf extracts possess high antifungal activity[13,43,44] (the hot water extract and the methanol extract were measured for their antifungal activity against *Arthrinium sacchari* M001 and *Chaetomium funicola* M002 strains).[44]

6. Antidiarrheal activity

P. guajava L. leaf has a long history of use as an antidiarrheal agent.[6,8] This activity could be explained through understanding the spasmolytic, antibacterial and antiamoebic activities, together with the following findings:

a. Quercetin was found to reduce the capillary permeability in the abdominal cavity.[34]

b. The alcoholic extract of the leaves possesses a morphine-like inhibition of acetyl choline release in the coaxially stimulated ileum, this morphine-like inhibition was found to be due to quercetin.[32]

c. The aqueous extracts produce a dose-related antidiarrheal effect. A dose of 0.2 ml/kg fresh leaf extract produced 65% inhibition of propulsion (inhibition of microlax-

Table 5: Antibacterial studies of *Psidium guajava* L. leaf.

Leaf extract		Activity	Ref.
Aqueous	*In vitro*	*Staphylococcus aureus*	9, 30, 37, 38
		β-streptococcus group A	30
		5 enterobacteria pathogenic to man (benefit in treatment of gastrointestinal disorders)	39
		Salmonella paratyphi (causative agent for enteric fever)	40
	In vivo	in mice infected by *H. strep "Richards"*	9
		Salmonella typhi infection in wistar rats (causative agent for enteric fever)	38, 41
		Causative agent for desentry (*Shigella dysenteriae, Shigella flexneri and Shigella sonnei*) and cholera (*Vibrio cholerae*).	38
Alcoholic	*In vitro*	*Neurospora crassa* and 20 other clinically important bacteria	40
	In vivo	in mice infected by *H. strep "Richards"*	9
50% ethanolic	*In vitro*	*E. coli* (constitute a feasible treatment option for diarrhea caused by *E. coli* or by *S. aureus*-produced toxins)	37
Methanolic	*In vitro*	*Staphylococcus aureus*	9, 30, 42, 43
		β-streptococcus group A	30
Chloroformic	*In vitro*	*Staphylococcus aureus*	9. 13, 30
		β-streptococcus group A	30
Acetonic	*In vitro*	*Staphylococccus aureus*	37, 43
		E. coli	
Essential oil	*In vitro*	*S. aureus*	42
		Salmonella sp.	

induced experimental diarrhea with narcotic like extracts of *Psidium guajava* leaf in rats).[45]

d. Oral administration of the methanol extract of the leaves reduces intestinal transit time and prevents castor oil-induced diarrhea in mice.[46]

e. Another study showed that the aqueous extract of the leaves significantly retarded the propulsion of charcoal meal and significantly inhibited the PGE_2-induced enteropooling.[47]

f. A recent study suggested that the antidiarrhoeal activity is through the inhibition of intracellular calcium release.[48]

7. Anticestodal activity

The leaf extract of *P. guajava* possesses anticestodal efficacy. Study supports its folk medicinal use in the treatment of intestinal-worm infections in northeastern part of India.[49]

8. Antidiabetic, hypoglycemic and anti-hyperlipidemic activities

Guava leaf has been used for the purpose of medical treatment of diabetes mellitus among people for many years.[50]

a. Reduction of postprandial blood glucose elevation:
Oral administration of a 50% ethanolic extract of the leaves and/or n-butanol soluble fraction from the ethanolic extract was found to inhibit the hyperglycemia in alloxan-induced diabetic rats. The active principles in the ethanolic and *n*-butanol extracts were identified as the tannins isostrictinin, strictinin, and pedunculagin,[16] while effective oral dose of the water extract which showed statistically significant hypoglycemic activity on alloxan-induced diabetic rats; in both acute and sub-acute tests was 250 mg/kg.[51]

b. Inhibition of alpha – glucosidase enzymes:
Guava tea manufactured from hot water extract of the leaves, have been confirmed to inhibit the activities of carbohydrate-degrading enzymes and to suppress the postprandial blood glucose level of human subjects.[52]

Single ingestion of guava extract can reduce postprandial glucose elevation via the inhibition of alpha-glucosidase in mice and human subjects with or without diabetes. Furthermore, it was found to have milder activity than voglibose.[53]

c. Improvement of diabetes symptoms, hyperlipidemia, hypercholesterolemia and hypoadeponectinemia.
It was shown that the consecutive ingestion of Guava Leaf Tea together with every meal improves not only hyperglycemia but also hypoadiponectinemia, hypercholesterolemia and hyperlipidemia in pre-diabetic and diabetic patients with or without hyperlipidemia. The consecutive ingestion also ameliorates high blood cholesterol level in subjects with hypercholesterolemia or borderline hypercholesterolemia.[53]

The leaf extract is also claimed as lipase inhibitors inhibiting carbohydrate absorption and preventing obesity, heart disease and atherosclerosis.[54]

Guava leaf extracts are potent antiglycation agents, which can be of great value in the preventive glycation-associated complications in diabetes.[55]

Thus, it is suggested that guava may be employed to improve and/or prevent the disease of diabetes mellitus.[53,56]

9. Antioxidant activity

Guava fruit is a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds.[57] The fruit is preferable as a dietary supplement for the prevention of atherosclerosis due to its content of polyphenols.[58]

Meanwhile the leaf was proven to have strong antioxidant activity,[59-61] since it possesses strong DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, potent inhibitory activity of lipid peroxidation and strong inhibition against oxidative cell death (H_2O_2-induced oxidative cell death)[59] and therefore guava could be used to extend the shelf life of foodstuffs, to reduce wastage and nutritional losses by inhibiting and delaying oxidation. As a conclusion, supplementing a balanced diet with guava leaf extracts may provide health-promoting effects.[60]

10. Cardioprotective effects of *Psidiuim guajava* L. leaves extract against ischemia-reperfusion injury in perfused rat hearts

P. Guajava L. possesses cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts, primarily through their radical-scavenging actions. The extract significantly attenuates ischemic contracture during ischemia and improves myocardial dysfunction after reperfusion. Quercetin and gallic acid also exerted similar beneficial effects.[62]

The study performed lately on the Egyptian plant showed that the alcoholic extract (400 μg/ml), aqueous extract (550 μg/ml), ethyl acetate (500 μg/ml) and essential oil (700 μg/ml) of the leaves inhibit the ventricular contraction of isolated rabbit heart. Isolated quercetin exhibits a dose related inhibition of ventricular contraction from

100 µg/ml.[6] Extracts of the leaf depress myocardial inotropism.[63]

Aqueous leaves extract of *Psidium guajava* significantly and dose-dependently (0.25-2 mg/ml) contracted aorta rings.[64]

11. Antimutagenic activity

P. guajava L. leaves possesses antimutagenic activity;[65,66] the leaves contain flavonoids which may be responsible for this activity and probably the anticarcinogenic properties of the leaves.[65]

(+)-gallocatechin isolated from the methanol extract of guava leaves was found to be a bio-antimutagenic compound against UV-induced mutation in *Escherichia coli*.[67]

Lately cytotoxic phenylethanol glycosides were isolated from the seeds.[68]

12. Relative retroviral reverse transcriptase inhibitory activity

At a concentration of 125 µg/ml, hot water extract showed 61% a relative retroviral reverse transcriptase inhibitory activity.[69]

13. Depressant activity on central nervous system

The methanol, hexane, ethyl acetate extracts of the leaves and the isolated sesquiterpenes, especially caryophyllene oxide and ß-selinene, which were by far the largest single components, exhibit a CNS depressant activity by potentiating the phenobarbitone sleeping time in mice.[18,46,70]

The essential oil and the aqueous extracts produce a moderate tranquillizing effect while ethyl acetate and alcoholic extracts are more active.[7]

14. The antinociceptive/analgesic activity

The hexane, ethyl acetate, methanol extracts of the leaves as well as the leaf essential oil were demonstrated dose-dependent antinociceptive effects.[46,70,71] The methanol extract also exhibits antipyretic effect,[46] meanwhile the aqueous extract was found to possess analgesic activity.[72]

15. Anti-inflammatory activity

The essential oil, the aqueous, the alcoholic, the methanolic and the ethyl acetate extracts, all produce a significant anti-inflammatory activity.[7,46,72]

Meanwhile benzophenone glycosides, sesquiterpenes, and flavonoids purified from the leaves are claimed as allergy inhibitors.[12]

16. Effect of guava extract on the arterial blood pressure

Guava extract following oral and intraperitoneal administration produces a significant reduction in arterial blood pressure without affecting the heart rate or respiratory rate.[73] A recent study suggested that the antihypertensive use in traditional medicine is through the inhibition of intracellular calcium release.[48]

17. Antiulcer activity

P.guajava possesses antiulcer activity acid secretion inhibitory effect in aspirin induced gastric ulcer model mediated through prostaglandins.[74]

18. Hepatoprotective activity

The aqueous extract of leaves of guava plant possesses good hepatoprotective activity.[75]

19. Other activities

a) The aqueous extract of the leaves, barks, and flowers were stated to have nicotinic receptor antagonist properties.[23]
b) The alcoholic extract (0.2 mg/100ml), aqueous extract (0.3 mg/100ml), ethyl acetate extract (0.4 mg%), essential oil (0.5 mg%) of the leaves and quercetin (0.5 mg%) produce a marked inhibition of isolated rat uterus.[7]
c) The leaf extract inhibits alpha-amylase activity and can be used as health beverage[76] and was found to inhibit the adherence of the early plaque settlers which include *Streptococcus mitis*, *Streptococcus sanguinis* and *Actinomyces* species. The mechanism of which may involve a modification of the hydrophobic bonding between the bacteria and the salivary components covering the tooth surfaces.[77]

TOXICITY OF GUAVA LEAF EXTRACT

The water extract of *P. guajava* leaves has no short term harmful effect,[39] and was found to be non toxic to rats and mice at a dose of 5g/Kg. i.e. LD_{50} was more than 5 g/kg.[30]

The study peformed on the Egyptian plant lately stated that the ethyl acetate extract is not toxic in doses up to 1.40 g/kg body weight, the alcoholic extract up to 2.05 g/kg, the aqueous extract up to 2.35g/kg and the essential oil up to 0.62 g/kg.[7]

Guava tea intake raises no changes in parameters of iron metabolism, liver and kidney functions and of blood chemistry data. In addition hypoglycemia is not caused by excess ingestion of guava tea.[54]

In single-dose and 1-month repeated dose toxicity studies, the oral administration of GvEx (200 and 2000 mg/kg/day) caused no abnormal effects in rats, indicating that there is neither acute nor chronic toxicity. Guava Leaf Tea had a lower mutagenic activity than commercial green tea and black tea in a DNA repair test (Rec-assay); however, these teas showed no mutagenic activity in a bacterial reverse mutation test (Ames test). Moreover, GvEx did not induce chromosomal aberrations in a micronuclear test using peripheral blood erythrocytes, which were prepared from mice by a single oral administration of GvEx (2000 mg/kg). From these findings, it is suggested that Guava Leaf Tea and these commercial teas have no genotoxicity.[53]

ASSAY

Quercetin content

Quercetin content of the powdered leaves (using the previously published method)[75]

1. Extract 2 g powdered Guava leaves with 20.0 ml of boiling water for 10 minutes, cool and filter.
2. To 10.0 ml of the previously prepared filtrate add 2 ml 25% HCl and heat on a water-bath for 25 minutes then cool and filter.
3. Extract this filtrate with 4 successive quantities, 25 ml each, of butanol.
4. Concentrate the combined butanol extracts under reduced pressure and redissolve in methanol adjusting the volume to 10.0 ml (volumetric flask). The resulting 10.0 ml is equivalent to 1 g powdered guava leaf.
5. Apply bands of this sample alongside with bands of standard quercetin (0.5 µg/µl) on 20 × 10 cm aluminium silica gel 60 F_{254} HPTLC plates by means of Linomat IV automated spray-on band applicator operated with the following settings: band length 6 mm, application rate 15 s/µl, distance between bands 4 mm, distance from the plate side edge 1.2 cm, and distance from the bottom of the plate 1.5 cm.
6. For construction of the calibration curve apply 1.00 µl, 2.00 µl, 3.00 µl, 4.00 µl, 5.00 µl and 6.00 µl of the quercetin standard (o.5 µg/µl). Apply each concentration trice and calculate the average.
7. Apply each sample as triplicate of 5.00 µl and then average is calculated.
8. Develop the plate to a distance of 6 cm beyond the origin with toluene-acetone-methanol-formic acid (46:8:5:1) in a vapour equilibrated Camag HPTLC twin trough chamber. After development, air dry the plates for 15 minutes.
9. Densitometrically quantify the bands using Camag TLC scanner 3 and WINCATS software. Operate the scanner in the Absorption/Reflection mode with 5 × 0.1 mm slit dimension, 20 mm/sec scanning rate and 20 nm monochromator bandwidth at an optimized wavelength 254 nm.
10. Calculate the concentration of quercetin in the sample with respect to the calibration curve constructed.
11. Quercetin concentration should not be less than 200 mg/ 100 g powdered leaf.

Quercetin content of the prepared extract

Proceed from step two from the assay of the powdered leaves.

Calculate the quercetin content of 1 ml of the extract.

Calculate the quercetin content of the powdered leaves from which the extract was prepared from.

Quercetin content of the final product

Proceed from step two from the assay of the powdered leaves.

Calculate the quercetin content of 1 ml of the final product.

APPENDIX I

Selection of the best solvent and method for the extraction of *Psidium guajava* L. leaves using quercetin as a marker compound

Samples of the same powdered guava leaves (2 g each) were extracted separately by 20.00 ml of water-ethanol in different proportions using maceration in order to determine the best water - ethanol ratio for extraction of the quercetin content.

Samples of the same powdered guava leaves (2 g each) were extracted separately by 20.00 ml of water using infusion and decoction (boiling under reflux for certain time) once for 10 minutes and once for 20 minutes.

Each extract was filtered and 10.00 ml of the extract was quantitatively estimated by the previously proposed method. Accordingly, the extract was hydrolysed using 2 ml of HCl, extracted with butanol, dried, redissolved in methanol adjusting the volume to 10.00 ml and this solution was finally quantified for its quercetin content using HPTLC with the same parameters in order to determine the best solvent that gives the highest yield of quercetin and its glycosides. The trials were conducted in triplicate. The details of the extraction procedures are given in table 6.

RESULTS

Results of triplicate trials are presented in table 7.

Table 6: Quercetin yeild of the leaves using different solvents and extraction methods.

Sample #	Solvent	Method of extraction	Concentration mg/100 g
1	Water	Infusion	138.99
2	Water	Decoction (boiling for 10 min)	181.87
3	Water	Decoction (boiling for 20 min)	185.13
4	16% Ethanol	Maceration for 24 hours	***255.98***
5	50% Ethanol	Maceration for 24 hours	185.46
6	70% Ethanol	Maceration for 24 hours	111.18
7	100% Ethanol	Maceration for 24 hours	89.65

Table 7: Samples of *P. guajava* L. leaves collected from different areas and their quercetin content.

Sample	Concentration mg/100 g
Tahrir (Alex. Cairo desert road at kilo 47)	286.667
Cairo	179.333
Fayoum	215.78
Anshas	380.65
Maamora	***392.7***
Edco	146.667
Agamy	215.45
Northern coast	327.5

Table 8: Samples of *P. guajava* L. leaves collected from trees at different stages f growth and their quercetin content.

Sample	Concentration of quercetin mg/100 g	
	Agamy	Maamora
Flowering stage	211.25	375
Premature fruiting	***215.45***	***392.7***
Mature fruiting	181.05	326.98
Post harvesting	199.34	336.86
Post harvesting and preflowering	206.1	374.6

APPENDIX II

Quercetin content of *P. guajava* L. leaves collected from different areas

Samples from different areas were collected from trees during the premature fruiting stage. The collected samples were analysed according to the previously proposed method in order to reveal the best location in which guava trees yield the highest quercetin content.

Samples of powdered guava leaf (2 g each) were extracted with 20.0 ml of water by decoction for 10 minutes and filtered. An aliquot (10.0 ml) was analysed using the previous method and the amount of quercetin content are presented in table 7.

Quercetin content of *P. guajava* L. leaves collected from trees at different stages of growth

Guava leaf samples were collected from the determined best 2 different areas (Agamy and Maamora) from trees at five different stages of growth, namely the flowering stage, the premature fruiting stage i.e. while fruits were small and green, the mature fruiting i.e. while the fruits were mature and yellow, post harvesting the fruits and post harvesting and preflowering. The collected samples are analysed for their quercetin content according to the proposed method in order to reveal the best stage of growth during which guava trees yield the highest quercetin content. The results are presented in table 8.

DISCUSSION

From table 6 it is obvious that the best solvent for extracting quercetin and its glycosides is the 16% ethanol and maceration for 24 hours, this is indicated by the highest quercetin percent (255.98 %). On increasing the strength of ethanol, the yield of quercetin was decreased, reaching a minimum yield with absolute ethanol (89.65 %). For the aqueous extraction the decoction is much better than the infusion. It is also obvious that 10 minutes decoction is very enough for the extraction with water and increasing the time does not increase significantly the amount extracted. From table 7 it is obvious that the highest quercetin content is during the premature fruiting stage. Nevertheless, and from the economic point of view, samples taken post harvesting of the fruits contain good amount of quercetin glycosides, as well. From table 8 it is obvious that samples collected from Maamora showed the best results. Samples collected from Anshas, Northern coast showed also good results.

REFERENCES

1. Pandey BP. Taxonomy of Angiosperms. 6th ed. New Delhi: S. Chand and Company Ltd; 1999; p. 227-33.
2. Nakason HY, Paull, RE. Tropical Fruits. New York: CAB international; 1998; p. 149-72.
3. Jackson DI, Looney, NE. Temperate and Subtropical Fruit Production. 2nd ed. New York: CABI Publishing; 1999; p. 267.
4. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: W. B. Saunders ltd; 2002.
5. WHO Regional Publications. Medicinal Plants in the South Pacific. Western Pacific Series No. 19. Western Pacific Region; 1998; p. 161.
6. Ross IA. Medicinal plants of the world: Chemical constituents, traditional and modern medicinal uses. New Jersey; Humana press: 1999.
7. Abdel Wahab SM, Hifawy MS, El Gohary HM, Isak, M. Study of Carbohydrates, Lipids, Protein, Flavonoids, Vitamine C and Biological

Activity of *Psidium guajava* L. Growing in Egypt. Egypt J Biomed Sci. 2004; 16:35-52.

8. Lozoya X, Meckes M, Abou-Zaid M, Tortoriello J, Nozzolillo C, Arnason JT. Quercetin glycosides in *Psidium guajava* L. leaves and determination of a spasmolytic principle. Arch med res. 1994; 25(1):11-5.
9. El Khadem H, Mohamed YS. Constituents of the Leaves of *Psidium guajava,* L. Part II. Quercetin, Avicularin, and Guajaverin. J Chem Soc. 1958; 3320-3.
10. Arima H, Danno G. Isolation of antimicrobial compounds from Guava (*Psidium guajava* L.) and their structural elucidation. Bioscience Biotechnology Biochemistry. 2002; 66(8):1727-30.
11. Seshadri T, Vasishta K. Polyphenols of the leaves of Psidium guava; quercetin, guaijaverin, leucocyanidin, amritoside. Phytochemistry. 1965; 4(6):989-992.
12. Kandil FE, El-Sayed NH, Micheal HN, Ishak MS, Mabry TJ. Flavonoids from *Psidium guajava*. Asian Journal of Chemistry. 1997; 9(4):871-2.
13. Metwalli AM, Omar AA, Harraz FM, El Sohafy SM. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacognosy Magazine. 2010; 6(23):216-22.
14. Okuda T, Hatano T, Yazaki K. Guavin B, an ellagitannin of a novel type. Chem Pharm Bull. 1984; 32(9):3787-8.
15. Okuda T, Yoshida T, Hatano T, Yazaki K, Ikegami Y, Shingu T. Guavins A, C and D, complex tannins from *Psidium guajava*. Chem Pharm Bull. 1987; 35(1):443-6.
16. Maruyama Y, Matsuda H, Matsuda R, Kubo M, Hatano T, Okuda T. Study on *Psidium guajava* L. (I). Antidiabetic effect and effective components of the leaf of *Psidium guajava* L. (Part 1). Shoyakugaku Zasshi(Japan). 1985; 39(4):261-9.
17. Matsuo T, Hanamure N, Shimoi K, Nakamura Y, Tomita I. Identification of (+)-gallochatechin as a bio-antimutagenic compound in Psidium guava leaves. Phytochemistry. 1994; 36(4):1027-9.
18. Meckes M, Calzada F, Tortoriello J, Gonzalez J, Martinez M. Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. Phtyother Res. 1996; 10(7):600-3.
19. Cueller Cueller A, Arteaga Lara R, Perez Zayas J. *Psidium guajava* L. Phytochemical screening and study of the essential oil. Rev Cubana Farm. 1984; 18(1):92-9.
20. Domingos a Silva J, Luz AIR, da Silva MHL, Andrade EHA, Zoghbi MB, Maia JG. Essential oils of the leaves and stems of four Psidium spp, Flavour and Fragrance Journal. 2003; 18(3):240-3.
21. Tucker AO, Maciarello MJ, Landrum LR. Volatile leaf oils of American Myrtaceae. III. *Psidium cattleianum* Sabine, *P. friedrichsthalianum* (Berg) Niedenzu, *P. guajava* L., *P. guineense* Sw., and *P. sartorianum* (Berg) Niedenzu. J Essent Oil Res. 1995; 7(2):187-90.
22. Fernandez X, Dunach E, Fellous R, Lizzani-Cuvelier L, Loiseau M, Dompe V, et al. Identification of thiazolidines in guava: stereochemical studies. Flavour and Fragrance Journal. 2001; 16(4):274-80.
23. Karawya MS, Abdel Wahab SM, Hifawy MS, Azzam SM, El Gohary HM. Essential Oil of Egyptian Guajava Leaves. Egypt J Biomed Sci. 1999; 40(2), 209.
24. Pino JA, Aguero J, Marbot R, Fuentes V. Leaf oil of *Psidium guajava* L. from Cuba. Journal of Essential oil Research. 2001; 13(1):61-2.
25. Begum S, Hassan SI, Siddiqui BS, Shaheen, F, Nabeel M, Gilani AH. Triterpenoids from the leaves of *Psidium guajava*. Phytochemistry. 2002; 61(4); 399-403.
26. Begum S, Hassan S, Ali S, Siddiqui B. Chemical constituents from the leaves of *Psidium guajava*. Natural Product Research. 2004; 18(2):135-40.
27. Varshney IP, Shamsuddin KM. Sapogenins of the leaves of *Psidium guajava*. Indian J Chem. 1964; 2(9):377-8.
28. Begum S, Hassan SI, Siddiqui, BS. Two new triterpenoids from the fresh leaves of *Psidium guajava*. Planta medica. 2002; 68:1149-52.
29. Prance GT, Kallunki JA, editors. Advances in Economic Botany: Ethnobotany in the neotropics. New York: New York Botanical Gardens; 1984; 9-23.
30. Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML, et al. Anticough and antimicrobial activities of *Psidium guajava* Linn. Leaf extract. J Ethnopharmacol. 1999; 67:203-12.
31. Lozoya X, Meckes M, Abou-Zaid M, Tortoriello J, Nozzolillo C, Arnason JT. Calcium-antagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L.. Arch med res. 1994; 25(1):17-21.
32. Lutterdodt GD. Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal disease. J Ethnopharmacol. 1989; 25(3):235-47.
33. Lozoya X, Becerril G, Martinez M. Model of intraluminal perfusion of the guinea pig ileum *in vitro* in the study of the antidiarrheal properties of the guava (*Psidium guajava*). Arch Invest Med (Mex). 1990; 21(2):155-62.
34. Zhang W, Chen B, Wang C, Zhu Q, Mo Z. Mechanism of Quercetin as antidiarrheal agent. Diyi Junyi Daxue Xuebao. 2003; 23(10):1029-31.
35. Lozoya X, Reyes-Morales H, Chávez-Soto M, Martínez-García M, Soto-González Y, Doubova SD. Intestinal anti-spasmodic effect of a phytodrug of *Psidium guajava folia* in the treatment of acute diarrheic disease. J Ethnopharmacol. 2002; 83(1-2):19-24.
36. Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, et al. Antiamoebic and spasmolytic activities of extracts from some antidiarrheal traditional preparations used in Kinshasa, Congo. Phytomedicine. 2000; 7(1), 31-8.
37. Vieira R, Rodrigues D, Gonçalves F, Menezes F, Aragao J, Sousa O. Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carya papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. Rev Inst Med trop S Paulo. 2001; 43(3):145-8.
38. Lutterodt GD, Ismail A, Basheer RH, Mohd Baharudin H. Antimicrobial effects of *Psidium guajava* L. extract as one mechanism of its antidiarrhoeal action. Malaysian Journal of Medical Sciences. 1999; 6(2):17-20.
39. Caceres A, Cano O, Samayoa B, Aguilar L. Plants used in Guatemala for the treatment of gastrointestinal disorders. Part I. Screening of 84 plants against enterobacteria. J Ethnopharmacol. 1990; 30(1):55-73.
40. Etuk EU, Francis UU. Acute Toxicity and Efficacy of *Psidium guajava* Leaves Water Extract on Salmonella Typhi Infected Wistar Rats. Pakistan Journal of Biological Sciences. 2003; 6(3):195-7.
41. Lopez-Abraham AM, Jimenez-Misas CA, Rojas-Hernandez NM. Biological activity of extracts of plants growing in Cuba. Part 1. Revista Cubana de Farmacia. 1980; 14(May-Aug):259-65.
42. Gonçalves FA, Andrade neto M, Bezerra J, Macrae A, Sousa OV, Fonteles-Filho A, et al. Antibacterial activity of Guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, *Xiphopenaeus kroyeri* (HELLER). Rev Inst Med trop S Paulo. 2008; 50(1):11-5.
43. Nair R; Chanda S. *In-vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. Brazilian Journal of Microbiology. 2007; 38:452-8.
44. Sato J, Goto K, Nanjo F, Kawai S, Murata K. Antifungal activity of plant extracts against Arthrinium sacchari and Chaetomium funicola. Journal of Bioscience and Bioengineering. 2000; 90(4):442-6.
45. Lutterodt GD. Inhibition of Microlax-induced experimental diarrhea with narcotic-like extracts of *Psidium guajava* leaf in rats. J Ethnopharmacol. 1992; 37(2):151-7.
46. Olajide OA, Awe SO, Markinde JM. Pharmacological studies on the leaf of *Psidium guajava*. Fitoterapia. 1999; 70(1):25-31.
47. Lin J, Puckree T, Mvelase TP. Anti-diarrheal evaluation of some medicinal plants used by Zulu traditional healers. J Ethnopharmacol. 2002; 79(1):53-56.
48. Belemtougri RG, Constantin B, Cognard C, Raymond G, Sawadogo L. Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and Diospyros mespiliformis L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. Journal of Zhejiang University Science B. 2006; 7(1):56-63.
49. Tangpu TV, Yadav AK. Anticestodal efficacy of *Psidium guajava* against experimental *Hymenolepis diminuta* infection in rats. Indian J Pharmacol. 2006; 38(1):29-32.
50. Sunagawa M, Shimada S, Zhang Z, Oonishi A, Nakamura M, Kosugi T. Plasma Insulin Concentration was Increased by Long-Term Ingestion of Guava Juice in Spontaneous Non-Insulin-Dependant Diabetes Mellitus (NIDDM) Rats. Journal of Health Science. 2004; 50(6):674-8.

51. Mukhtar HM, Ansari SH, Ali M, Naved T, Bhat ZA. Effect of water extract of *Psidium guajava* leaves on alloxan-induced diabetic rats. Pharmazie. 2004; 59(9):734-5.
52. Deguchi Y, Osada K, Chonan O, Kobayashi K, Ohashi A, Kitsukawa T, et al. Effectiveness of consecutive ingestion and excess intake of guava leaves tea in human volunteers. Nippon Shokuhin Shinsozai Kenkyukaishi. 2000; 3(1):19-28.
53. Deguchi Y, Miyazaki K. Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutr Metab (Lond). 2010; 7:9. doi:10.1186/1743-7075-7-9.
54. Yamanobe Y, Hattori F, Shimomura K. Extracts from Uncaria gambier, *Psidium guajava*, and Filipendula as lipase inhibitors. Jpn. Kokai Tokkyo Koho. Patency number JP 2000103741, Kind A2, Date 20000411, Application number JP 1998-277896, Date 19980930, 3 pp. (Japan).
55. Ju-Wen Wu, Chiu-Lan Hsieh, Hsiao-Yun Wang, Hui-Yin Chen. Inhibitory effects of guava (*Psidium guajava* L.) leaf extracts and its active compounds on the glycation process of protein. Food Chemistry. 2009; 113(1):78-8.
56. Cheng JT, Yang RS. Hypoglycemic effect of guava juice in mice and human subjects. Am J Chin Med. 1983; 11(1-4):74-6.
57. Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F. Guava Fruit (*Psidium guajava* L.) as a New Source of Antioxidant Dietary Fiber. Journal of Agricultural and Food Chemistry. 2001; 49(11):5489-93.
58. Gorinstein S, Zemser M, Haruenkit R, Chuthakorn R, Grauer F, Martin-Belloso O, et al. Comparative content of total polyphenols and dietary fiber in tropical fruits and persimmon. Journal of Nutritional Biochemistry. 1999; 10(6):367-71.
59. Masuda T, Oyama Y, Inaba Y, Toi Y, Arata T, Takeda Y, et al. Antioxidant-related activities of ethanol extracts from edible and medicinal plants cultivated in Okinawa, Japan. Nippon Shokuhin Kagaku Kogaku Kaishi. 2002; 49(10):652-61.
60. Qian He, Nihorimbere V. Antioxidant power of phytochemicals from *Psidium guajava* leaf. Journal of Zhejiang University, Science. 2004; 5(6):676-683.
61. Hui-Yin Chen, Gow-Chin Yen. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chemistry. 2007; 101(2):686-94.
62. Yamashiro S, Noguchi K, Matsuzaki T, Miyagi K, Nakasone J, Sakanashi M, et al. Cardioprotective Effects of Extracts from *Psidium guajava* L. and *Limonium wrightii*, Okinawan Medicinal Plants, against Ischemia-Reperfusion Injury in Perfused Rat Hearts. Pharmacology. 2003; 67(3):128-35.
63. Conde Garcia EA, Nascimento VT, Santiago Santos AB. Inotropic effects of extracts of *Psidium guajava* L. (guava) leaves on the guinea pig atrium. Braz J Med Biol Res. 2003; 36(5):661-8.
64. Olatunji-Bello II, Odusanya AJ, Raji I, Ladipo CO. Contractile effect of the aqueous extract of *Psidium guajava* leaves on aortic rings in rat. Fitoterapia. 2007; 78(3):241-3.
65. Teixeira R, Camparoto M, Mantovani M, Vicentini V. Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L. in *in vitro* and *in vivo* assays. Genetics and Molecular Biology. 2003; 26(4):551-5.
66. Grover IS, Bala S. Studies on antimutagenic effects of guava (*Psidium guajava*) in Salmonella typhimurium. Mutant Re. 1993; 300(1),1-3.
67. Matsuo T, Hanamure N, Shimoi K, Nakamura Y, Tomita I. Identification of (+)-gallochatechin as a bio-antimutagenic compound in Psidium guava leaves. Phytochemistry. 1994; 36(4):1027-9.
68. Salib J, Michael H. Cytotoxic phenylethanol glycosides from *Psidium guajava* seeds. Phytochemistry. 2004; 65:2091-3.
69. Suthienkul O, Miyazaki O, Chulasiri M, Kositanont U, Oishi K. Retroviral reverse transcriptase inhibitory activity in Thai herbs and spices: screening with Moloney murine leukemia viral enzyme. Southeast Asian J Trop Med Public Health. 1993; 24(4):751-5.
70. Shaheen HM, Ali BH, Alqarawi AA, Bashir AK. Effect of *Psidium guajava* leaves on some aspects of the central nervous system in mice. Phytother Res. 2000; 14(2):107-11.
71. Somichit MN, Sulaiman MR, Ahmad Z, Israf DA, Hosni H. Non-opioid anti-nociceptive effect of *Psidium guajava* leaves extract. Journal of Natural Remedies. 2004; 4(2):174-8.
72. Ojewole JAO. Anti-Inflammatory and analgesic effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extracts in rats and mice. Methods Find Exp Clin Pharmacol. 2006; 28(7):441-6.
73. Lutterodt GD, Maleque A. Effects on mice locomotor activity of a narcotic-like principle from *Psidium guajava* leaves. J Ethnopharmacol. 1988; 24:219-31.
74. Das S, Dutta S, Deka S. A study of the anti-ulcer activity of the ethanolic extract of the leaves of *Psidium guajava* on experimental animal models. The Internet Journal of Pharmacology. 2009; 7(1).
75. Roy CK, Kamath JV, Asad M. Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. Indian J Exp Biol. 2006; 44(4):305-11.
76. Aoki A, Kudo T, Harada K, Makino T, Nagata K, Deguchi Y, et al. Clarification of *Psidium guajava* health beverage. . Jpn Kokai Tokkyo Koho. Patency number JP 2003000208, Kind A2, Date 20030107, Application number JP 2001-190751, Date 20010625, 7 pp. (Japan).
77. Abdul Razik F, Abd Rahim Z. The anti-adherence effect of *Piper betle* and *Psidium guajava* extracts on the adhesion of early settlers in dental plaque to saliva-coated glass surfaces. Journal of Oral Science. 2003; 45(4):201-6.
78. El Sohafy SM, Metwalli AM, Harraz FM, Omar AA. Quantification of flavonoids of Psidium guajava L. preparations by Planar Chromatography (HPTLC). Pharmacognosy Magazine. 2009; 4(17):61-6.

Utilization of Conventional COT System to facilitate Phytochemical and Pharmacological Studies: A Proposal

A.B.D. Selvam

Pharmacognosy Section, Botanical Survey of India, Howrah – 711103, India

ABSTRACT

The different colours that we visualize through our Eyes, the diverse odours that are sensed by the olfactory system of our Nose and the six sorts of basic tastes that are recognized by the taste buds of our Tongue, when we study the plant parts such as roots, stems, barks, leaves, flowers, fruits, seeds etc. are mainly due to the chemical constituents that are present in plants/plant parts. The Colour, Odour and Taste that are recorded from drug plants/plant parts through Organoleptic studies (one of the pharmacognostic parameters), are in fact the baseline information, based on which one can proceed further for a preliminary/detailed phytochemical and pharmacological screening in any plant/plant part. To highlight the importance of the above-mentioned features, a new system (COT system) has been proposed. COT is a three-letter acronym, which refers to Colour, Odour and Taste of the drug plants that could be used to figure out the nature of chemical compounds and their possible pharmacological activities.

Key words: COT system, Colour, Odour, Taste, Pharmacognosy, Phytochemistry, Pharmacology.

INTRODUCTION

'Face is the index of the Mind' says a proverb. It is true that our face reflects our heart, mind, mood and innermost feelings. This proverb is not only applicable for humanity, but also to the entire plant community as well. The nature of chemical compounds/active principles that are embedded within the plants/plant parts are being revealed through three different ways/forms viz. colour, odour and taste. In short, the colour, odour and taste are the indices or indicators of chemical constituents that are present in plants.

An acronym 'COT' has been coined in line with the proposed study by taking the first letter of the following words, Colour, Odour and Taste. The new acronym COT was coined following a famous acronym ENT (Ear, Nose and Throat), which is one of the familiar branches of medicine and surgery that specializes in the diagnosis and treatment of Ear, Nose and Throat disorders/diseases.

Address for correspondence:
E-mail: abd_selvam@yahoo.co.in

DOI: 10.5530/pj.2011.21.18

Evaluation of Colour, Odour and Taste (COT), is an essential part of organoleptic studies (one of the pharmacognostic parameters), which deals with study of drug plants/plant parts using our sense organs such as Eyes, Nose and Tongue respectively.

Among the different pharmacognostic parameters, Organoleptic study is the only method/parameter that involves neither scientific instruments nor any expenses.[1] The evaluation of the crude drug is an essential part of pharmacognosy. The individual drug plant would undergo some procedures including organoleptic evaluation to determine various structural and functional features.[2]

The term organoleptic literally means impression on sense organs viz. eyes, nose, tongue, ears and touch. The organoleptic study is a qualitative method where worker applies sight, smell, taste, touch and hearing to record the characteristic features of crude drugs.[3] The proposed COT system is a conventional method for assessing/diagnosing the phytochemicals and their possible pharmacological actions that the plants/plant parts are bestowed with. The proposed COT system/method will abet the phytochemists and pharmacologists to proceed further for modern scientific studies to certain extent.

COT SYSTEM IN A NUTSHELL

Organoleptic (sensory) characters are useful in the evaluation of crude drugs. Organoleptic parameters such as Colour, Odour and Taste are very much useful to identify certain plants. The medicinal plants and their extracts possess characteristic odour and taste, which indicate the presence of Phytochemicals that are present in them.[4]

The colour of the drugs are standardized and determined by the "Inter-Society Color Council-National Bureau of Standards". The external colour of vegetable crude drugs varies from white to yellowish grey, brown, orange or brownish black. The odour of a drug may be either distinct (characteristic) or indistinct. The pharmacognostic terms that are generally used to define odour are aromatic, balsamic (resembling balsam), spicy (having the aroma of spice), alliaceous (smelling like onion or garlic), camphoraceous (smelling like camphor), terebinthinate (having the qualities of turpentine) and others. Taste is a sensation produced by certain substances that meet the taste buds located on the surface of the tongue. The taste may be sour (acidic), saline (salt like), saccharine (sweetish), bitter, alkaline and metallic.[5,6]

The appealing appearance (colours) of different plants/plant parts could be noticed from a little distance. The odour, fragrance or smell is normally felt when we approach the plants/plant parts reasonably nearby. Taste could be analyzed only when we chew it or taste it. In short, the chemical compounds that are present in plants give the colour, odour and taste to plants/plant parts; without phytochemicals, the plants will be colourless, odourless and tasteless. The data derived through COT system/method is in fact the baseline/preliminary information, based on which one can look for specific chemical constituents and pharmacological properties.

Since a number of people deal with medicinal plants, say for example, botanists, chemists, pharmacognosists, pharmacologists etc., the baseline information derived from COT system would be much useful to everyone.

Colour

The colour plays a major role in the identification of plant samples and also to understand the nature of phytochemicals that may be present in them. Not many organic compounds are coloured. Therefore, a coloured sample may be characteristic of certain groups of substances or functional group present in the unknown compound. The colour of the compound is attributed to the presence of chromophoric groups such as $-NO_2$, $-NH_2$, C=O, N=N etc.[7]

Odour

Since a large number of the organic compounds possess characteristic odours, it is expected that olfaction may disclose the presence of certain groups of substances. Except aromatic carboxylic acids, other compounds such as alcohols, esters, aldehydes, amines and ketones possess odour. Depending on the experience of the worker, it may be possible to relate the unknown to a certain class of compounds, for instance, esters have family fruity odour while aromatic hydrocarbons possess characteristic aroma.[7]

Taste

Taste is based on the reactions of human senses when test materials (drug plants/plant parts) come to contact with our tongue.[4] The characteristic taste that the medicinal plants and their extracts possess is an indication of the presence of a particular type/group of phytochemicals that are present in them. One of the oldest languages in the world, the Classical Tamil classifies the tastes into six primary categories viz. kaippu (bitter), thiththippu (sweet), pulippu (sour), uvarppu (salty), thuvarppu (astringent) and karppu (Pungent). The medicinal literature of classical Tamil (Siddha System of Medicine) also endorses the aforesaid tastes.[8]

An outline of the proposed COT system is furnished here under for understanding of the readers and experts.[9]

Organo-leptic characters	Components of COT system	Presence of possible Phytochemicals	Presence of possible Pharmacological activities
Colour	Yellow	Flavonoids	Some of the activities attributed to flavonoids are anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral.
	Orange-red, bright orange, red, deep red, brown, yellow	Carotenoids (pigments)	Carotenoids work against disorders such as cancer, heart disease and degenerative eye disease. Also, act as antioxidants.
	Blue, purple red, red	Anthocyanins (pigments)	Anthocyanins show effect on cardiovascular health, anti-cancer and anti-inflammatory properties.
	Yellowish, light grey to grey-green	Anthraquinones (aromatic compounds)	Anthraquinones have a laxative effect on the body. Also, act as antimalarials and antineoplastics.
	Yellowish, light brown, brown, black	Tannins	Tannins show antiseptic, antiviral, antibacterial and antiparasitic effects.

Continued . . .

Organo-leptic characters	Components of COT system	Presence of possible Phytochemicals	Presence of possible Pharmacological activities
	Green	Chlorophyll (pigments)	Cleanses the bloodstream, deodorizes bad breath and body odor, deactivates carcinogenic substances and halts tooth decay. It also shows wound healing and antioxidant properties.
Odour	Aromatic	Terpenoids (essential oils)	Strongly anti-septic, anti-inflammatory.
	Balsamic	Oleo-resin, resin	Possess antioxidant, anti-inflammatory, antimicrobial and antiarthritic activities
	Spicy	Eugenol extracted from Cloves.	Eugenol has pronounced antiseptic and anaesthetic properties.
	Alliaceous	Organosulfur compounds e.g. Allicin	Allicin exhibits antibacterial and anti-fungal properties.
	Camphoraceous	Terpenoid (Camphor)	Camphor used as an insect repellents and inhalants. Also, acts as slight local anesthetic and antimicrobial substance.
	Terebinthinate	Oleoresin (Turpentine)	Possesses antimicrobial, antioxidative, antimutagenic etc.
Taste	Bitter	Alkaloids	Alkaloids show wide range of actions including toxics, sedatives, anti-cancer, anti-inflammatory, reducing spasms, relieving pain etc.
	Sweet	Sugar compounds e.g. *Glycyrrhizine*, a triterpenoid glycoside	The main constituent of *Glycyrrhiza glabra* L., is *Glycyrrhizin*, which has antiviral, anti-inflammatory, anti-hepatotoxic, anti-allergic and anti-oxidative actions.
	Sour	Acids	Acids from citrus fruits possess antioxidant, anti-aging, anticancer, anti-inflammatory and disinfectant properties.
	Salty	Plant salt substitute (PSS)	Plant extracts of *Salicornia herbacea L.* (Chenopodiaceae) and *Laminaria japonica* Areschoug (Laminariaceae) may act as low sodium replacer, which reduces the risk of heart diseases.
	Astringent	Tannins	Tannins show antiseptic, antiviral, antibacterial properties etc. Tannin containing plant parts unpalatable to insects and grazing animals.
	Pungent	Organo-sulfur compounds (OSCs).	Organo-sulfur compounds isolated from chilly, ginger, garlic, onion, pepper, peppermint etc. possess antioxidant, anti-inflammatory, anti-cancerous, anti-microbial activities etc. It also reduces blood pressure.

CONCLUSION

Organoleptic evaluation of vegetable crude drugs is often not given much importance by pharmacognosists, which is in fact the pedestal for pharmacognostic, phytochemical and pharmacological studies. In this article, the usefulness of organoleptic characters mainly Colour, Odour and Taste have been highlighted, which would aid phytochemical and pharmacological studies. Further, it would also save manpower, time and money spent on screening the unknown phytochemicals and their pharmacological actions to a greater extent.

The Organoleptic data furnished in this article in a tabular form is only an outline. Further, it is suggested that a comprehensive data using the proposed COT system have to be prepared with the help of experts from three different disciplines such as pharmacognosy, phytochemistry and pharmacology and are made available to the aspiring academicians and researchers to facilitate their research work. It is also suggested that the proposed COT system should be popularized among the students, research scholars and others, especially those who deal with pharmacognosy, ethno-botany, phytochemistry and pharmacology for better utility.

REFERENCES

1. Selvam A. Can the term Phytosensology be preferred over the term Organoleptic?. Phcog Res [serial online] 2010 [cited 2011 Mar 30]; 2:271-2. Available from: http://www.phcogres.com/text.asp?2010/2/4/271/69131
2. herbdatanz.com [homepage on the Internet]. New Zealand: Herbdata Library; c2002-2008 [updated continually]. [about 7 p.]. Available from: http://www.herbdatanz.com/element_of_herbal_pharmacy.htm
3. Selvam A. Can the term organoleptic be considered equivalent to zoological? Phcog Mag 2006; 2(8):200-201. Available from: http://www.phcog.com/showbackissue.asp?issn=0973-1296;year=2006;volume=2;issue=8;month=October-December
4. Ismail Z, Islam S, Ahmad MN, Othman AR, Shakaff AYM. Organoleptic assessment of Centella asiatica by Taste sensor. J. Trop. Med. Plants. 2000; 1:27-31.
5. Ali M. Text Book of Pharmacognosy. New Delhi: CBS Publishers & Distributors; 2006.
6. Ansari SH. Pharmacognosy. Delhi: Birla Publications; 2006.
7. Raaman N. Phytochemical Techniques. New Delhi: New India Publishing Agency; 2006.
8. Anonymous. Lifco's Tamil-Tamil-English Dictionary. Chennai: The little flower company; 1996.
9. Andrew, Chevallier, Mnimh. The encyclopedia of Medicinal plants. London: Dorling Kindersley; 1996.