Pharmacognostical And Phytochemical Studies Of *Strychnos Potatorum* Linn Seeds

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ABSTRACT:

Introduction: Strychnos potatorum Linn (Fam: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lanka and Burma. In traditional system of medicine the seeds are used for the treatment of various ailments like jaundice, bronchitis, diabetes, conjunctivitis, chronic diarrhoea, dysentery etc. They are also used to clear muddy water by its coagulant action. Although the seeds are useful in various treatments, its pharmacognostic features and phytochemical analysis were not studied.
Methods: The pharmacognosy of the seed was studied by evaluating the macroscopic and microscopic characters, whereas the phytochemistry was studied by fractionation and HPTLC fingerprinting.
Results: Microscopic evaluation of the seed revealed the presence of testa with tangentially elongated shrunken parenchyma in the outer zone and trichome zone in the inner layer. Calcium oxalate crystals were seen on the surface of the seed. Endosperm tissue consists of palisade like epidermal cells, thick and prominent cuticle on the surface of the epidermis. Physiochemical constants of the seed powder showed 7.65% moisture, 4.39% alcohol soluble extractive, 12.25% water soluble extractive, 1.43% total ash, and 0.09% acid insoluble ash. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, steroids/triterpenes, polyphenolics, reducing sugars, saponins and anthocyanins. HPTLC screening of the ether fraction of SPP showed seven peaks, unsaponifiable fraction showed five peaks and alkaloidal fraction showed eight peaks at 260 nm.
Conclusion: The pharmacognostical features and physiochemical constants of *Strychnos potatorum* reported here will be useful to identify the seeds from its adulterants.

Keywords: Strychnos potatorum; pharmacognostical studies, physiochemical constants, HPTLC.

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INTRODUCTION

Strychnos potatorum Linn (Fam: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Srilanka and Burma[1]. In traditional system of medicine, the seeds are used in the treatment of gonorrhoea, leucorrhoea, gastropathy, bronchitis, chronic diarrhoea, dysentery, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, ulcers and other eye disease[2]. The ripe seeds are used for clearing muddy water. The clarification is due to the combined action of colloids and alkaloids in the seeds[3].

Phytochemical studies revealed the presence of diaboline (major alkaloid) and its acetate[4]; brucine, loganin, mannose, sucrose, arachidonic, lignoceric, linoleic, oleic, palmitic and stearic acids[5]; on saponification of the oil: β sitosterol, stigmasterol (also in leaves and bark along with campesterol); oleanolic acid and its 3β acetate, saponins containing oleanic acid, galactose and mannose (seeds)[6]; triterpenes and sterols[7] mannogalactans[8,9,10].

The total alkaloid fraction isolated from the seeds of *Strychnos potatorum* when administered in mice and rats at the doses of 70–100 mg/kg, i.p. produced restlessness, irritability and tremors, followed by convulsions of tonic type all over the body[11] and hypotensive action[12]. The methanolic extract of the dried seeds was found to have antidiarrhoel[13] and diuretic[14] activities. The seed powder was found to possess antidiabetic activity[15]. Mannogalactans isolated from the seeds of *Strychnos potatorum* showed antihypercholesterolemic activity in experimental rats[8].

MATERIALS AND METHODS

Plant material

The seed specimens for the study were collected from crude drug market, Chennai and the genuinity of the seed specimen was confirmed by Dr. S. Jayaraman, Botanist, Plant Anatomy Research Centre, Chennai, Tamilnadu. The following protocol was used for the pharmacognostic authentication.

Macroscopic evaluation

The seeds were evaluated and its macroscopy was photographed to view its special characteristics like shape, size and colour.

Microscopic evaluation

i) Processing of the specimen

The seed specimens were boiled in distilled water for about 30 min in order to soften the tissues and fixed in FAA (Formalin 5 ml + acetic acid + 70% ethyl alcohol 90 ml). After 24 hrs of fixing, the specimen was dehydrated with graded series of tertiary butyl alcohol. Infiltration of the specimen was carried by gradual addition of paraffin wax until, tertiary butyl alcohol solution attained super saturation[16]. The specimens were cast into paraffin blocks.

ii) Sectioning

The paraffin embedded specimens were sectioned to a thickness of 10–12 μm and stained with toluidine blue[17]. For studying the epidermal trichomes, the seeds were immersed in hot water for few min and the trichomes were removed by scrapping the surface of the seed with the scalpel. The scrapped material was mounted in a drop of glycerin and sealed with the cover slip.

iii) Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Calcium oxalate crystals and trichomes were studied under polarized light and photographed.

Physiochemical constants

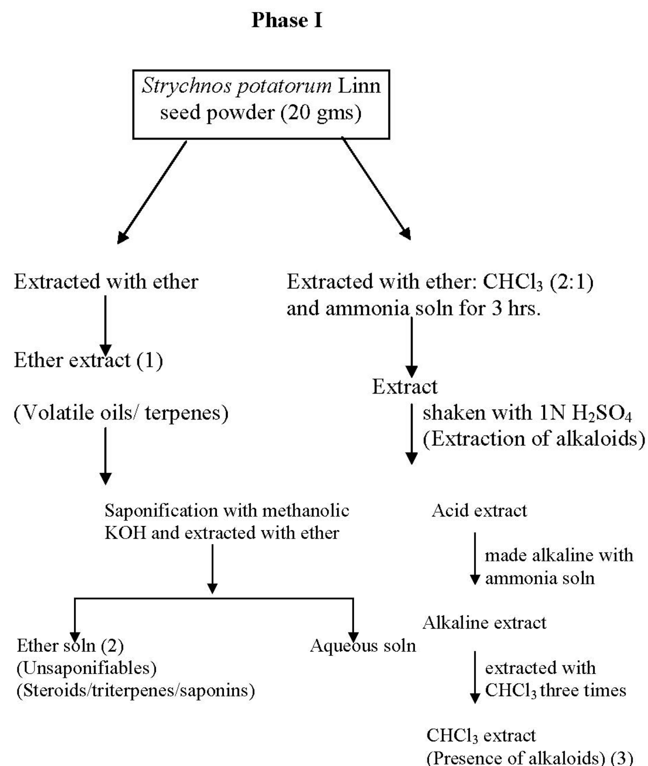
Determination of moisture content, extractive values (Alcohol and water soluble extractives) and ash values (Total ash, acid insoluble ash and water soluble ash) were done according to the methods given in Ayurvedic Pharmacopoeia of India, 1999[18].

Phytochemical analysis

Fractionation

The seed powder (SPP) was processed to obtain different fractions and used for preliminary qualitative chemical analysis. The TLC and HPTLC patterns of specific fractions were also developed.

The seed powder was processed as follows:



Phase II

Powdered *Strychnos potatorum* seeds was extracted with methanol for5 min on a water bath at about 60°C and then filtered. The methanol extract (4) was tested for alkaloids, flavonoids and tannins. The marc obtained was extracted with hot water. The water extract (5) was tested for sugars, tannins etc.

Preliminary qualitative phytochemical screening was carried out according to the method of Kokate, 1997[19] and Trease and Evans, 1983[20].

TLC analysis

Thin layer chromatography was developed in precoated silica gel plates Merck 60 F_{254} of 0.2mm thickness.

SPP fractions

i) Ether extract

Solvent systems: Hexane: Ethyl acetate (9: 1).
Detection: Vanillin sulphuric acid

ii) Unsaponifiables in ether

Solvent system: Toluene: Ethyl acetate (4: 1).
Detection: UV at 260 nm, Libermann burchardt reagent,

iii) $CHCl_3$ extract (Alkaloid fraction)

Solvent system: Toluene: Ethyl acetate: Diethyl amine (7: 2: 1).
Detection: Under UV at 260 nm

High-pressure thin layer chromatographic study (HPTLC)

The phytoconstituents identified through qualitative chemical analysis and TLC was further processed for HPTLC in precoated silica gel plates (Merck 60F254) of 0.2 mm thickness and 10×10 cm plate size. About 3 µl of the samples were applied in the plates using Linomat IV Automatic Spotter. Then the plates were developed in CAMAG Twin Trough Chamber of dimension 20×10 cm. The developed plates were air-dried and detected by UV (under deuterium lamp, 260 nm) / spraying reagents (under tungsten lamp, 550 nm) and densitometric scanning was done using CAMAG TLC scanner to record the peaks. The Rf values and the percentage area of the separated phytoconstituents were determined.

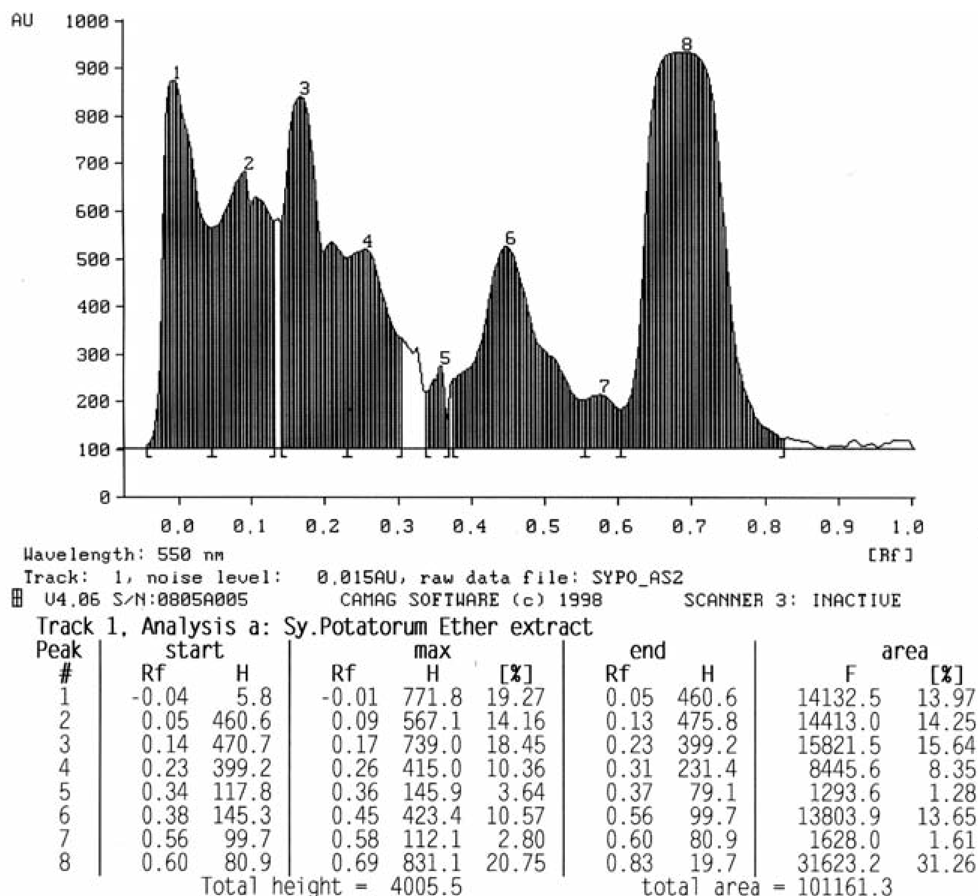
RESULTS AND DISCUSSION

Macroscopic features of the seed

The gross morphology of the unground whole seed was circular in surface view and ellipsoidal in lateral view. The edge of the seed is marked by thin, smooth, even circular ridge. The hilum occurs at the center of the seed and appears as a minute scar. The micropyle lies on the median portion of the lateral ridge. The seeds are ash grey and measure 7 mm in diameter, and 5 mm in thickness. The seed surface is smooth and even. No specific odour or taste is evident. The seeds are hard and strong, become soft on prolonged boiling. The surface of the dry seed exhibited fine reticulate marking which are visible only to the hand-lens. (Fig.1)

Microscopic features (Figs. 2, 3, 4 & 5)

This is a valuable test for both powders and for unground drugs. The identity of many adulterants of unground drugs can be established or confirmed by an examination

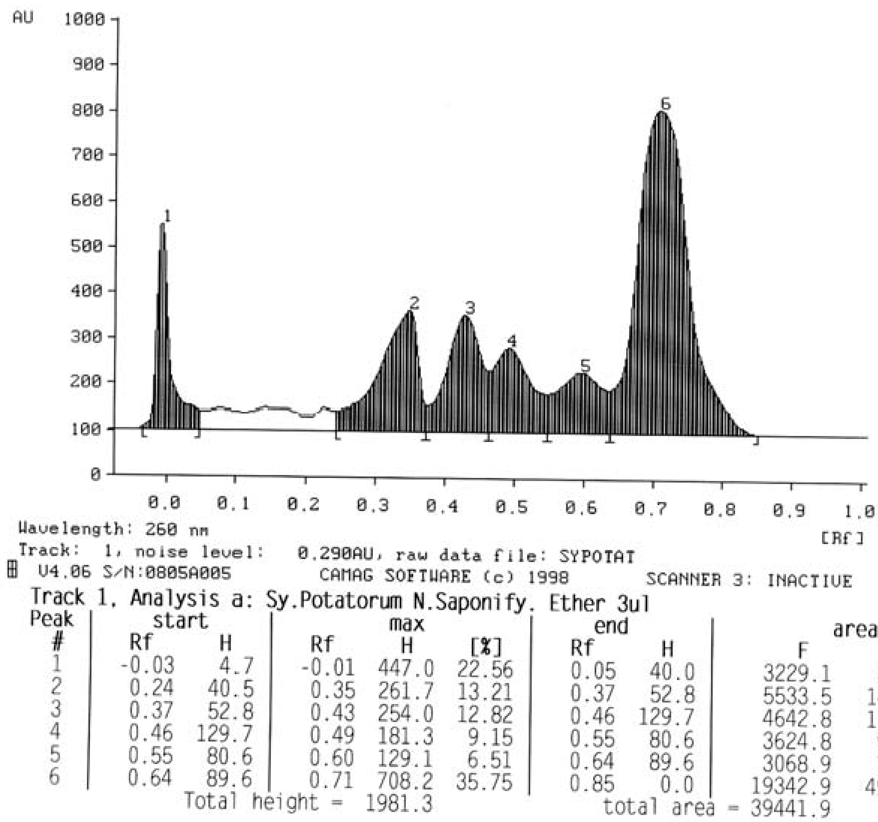


Track 1, Analysis a: Sy.Potatorum Ether extract

Peak	start		max			end		area	
#	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	-0.04	5.8	-0.01	771.8	19.27	0.05	460.6	14132.5	13.97
2	0.05	460.6	0.09	567.1	14.16	0.13	475.8	14413.0	14.25
3	0.14	470.7	0.17	739.0	18.45	0.23	399.2	15821.5	15.64
4	0.23	399.2	0.26	415.0	10.36	0.31	231.4	8445.6	8.35
5	0.34	117.8	0.36	145.9	3.64	0.37	79.1	1293.6	1.28
6	0.38	145.3	0.45	423.4	10.57	0.56	99.7	13803.9	13.65
7	0.56	99.7	0.58	112.1	2.80	0.60	80.9	1628.0	1.61
8	0.60	80.9	0.69	831.1	20.75	0.83	19.7	31623.2	31.26

Total height = 4005.5 total area = 101161.3

Spectrum 1: HPTLC fingerprinting of ether fraction of SPP.

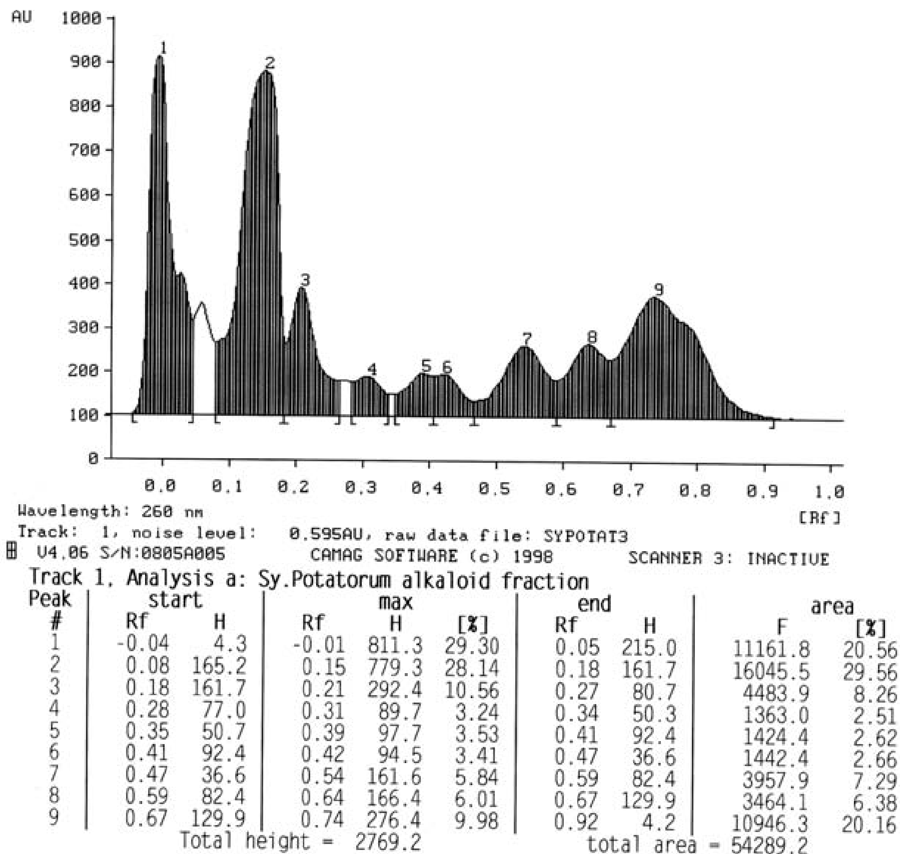


Track 1, Analysis a: Sy.Potatorum N.Saponify. Ether 3ul

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	-0.03	4.7	-0.01	447.0	22.56	0.05	40.0	3229.1	8.19
2	0.24	40.5	0.35	261.7	13.21	0.37	52.8	5533.5	14.03
3	0.37	52.8	0.43	254.0	12.82	0.46	129.7	4642.8	11.77
4	0.46	129.7	0.49	181.3	9.15	0.55	80.6	3624.8	9.19
5	0.55	80.6	0.60	129.1	6.51	0.64	89.6	3068.9	7.78
6	0.64	89.6	0.71	708.2	35.75	0.85	0.0	19342.9	49.04

Total height = 1981.3 total area = 39441.9

Spectrum 2: HPTLC fingerprinting of Unsaponifiable fraction of SPP.



Track 1, Analysis a: Sy.Potatorum alkaloid fraction

Peak #	start Rf	start H	max Rf	max H	max [%]	end Rf	end H	area F	area [%]
1	-0.04	4.3	-0.01	811.3	29.30	0.05	215.0	11161.8	20.56
2	0.08	165.2	0.15	779.3	28.14	0.18	161.7	16045.5	29.56
3	0.18	161.7	0.21	292.4	10.56	0.27	80.7	4483.9	8.26
4	0.28	77.0	0.31	89.7	3.24	0.34	50.3	1363.0	2.51
5	0.35	50.7	0.39	97.7	3.53	0.41	92.4	1424.4	2.62
6	0.41	92.4	0.42	94.5	3.41	0.47	36.6	1442.4	2.66
7	0.47	36.6	0.54	161.6	5.84	0.59	82.4	3957.9	7.29
8	0.59	82.4	0.64	166.4	6.01	0.67	129.9	3464.1	6.38
9	0.67	129.9	0.74	276.4	9.98	0.92	4.2	10946.3	20.16

Total height = 2769.2 total area = 54289.2

Spectrum 3: HPTLC fingerprinting of alkaloidal fraction of SPP.

Figure 1 Macroscopic features of the seeds. H – Hilum; LV – Lateral View; M – Micropyle; SV – Side View of the seed.

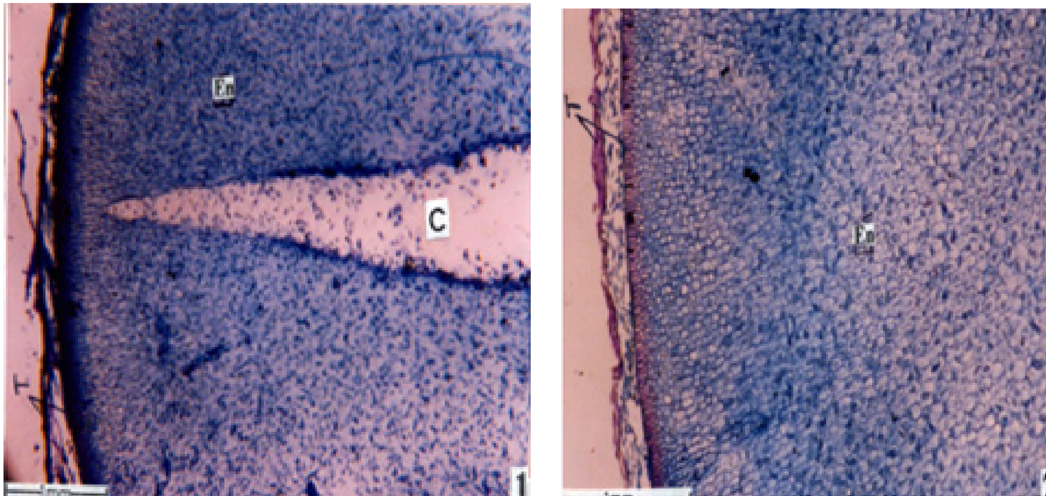


Figure 2 Microscopic features of the seeds. Sectional view of the seeds. C – Central cavity; En – Endosperm ; T – Testa.

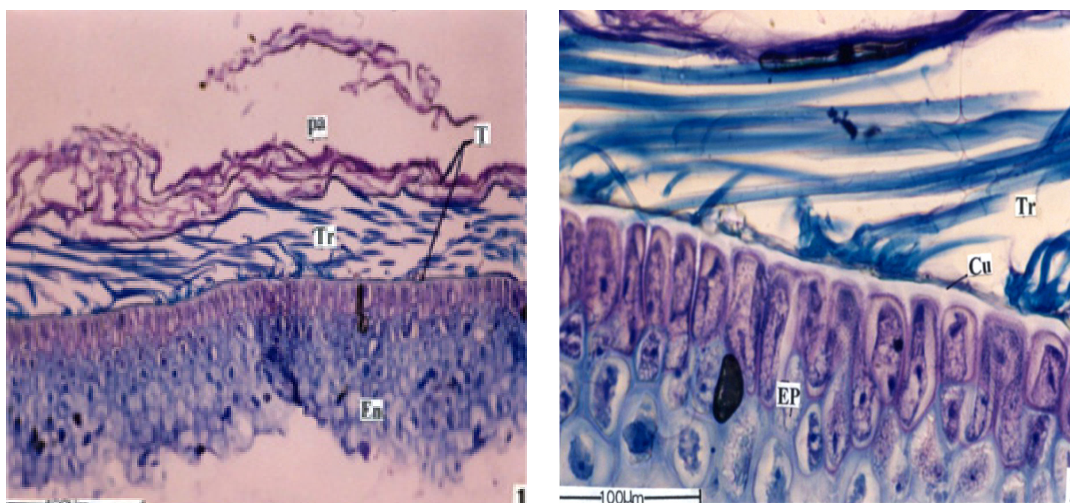


Figure 3 TS of the seeds to show enlarged view of the Testa and endosperm. 1. A portion of the Testa and Endosperm. 2. Basal part of the trichomes with horizontal part and epidermis of the endosperm. Cu – Cuticle of the epidermis; En – Endosperm; EP – Epidermes; Pa – Parenchyma zone; T- Testa ; Tr – Trichome.

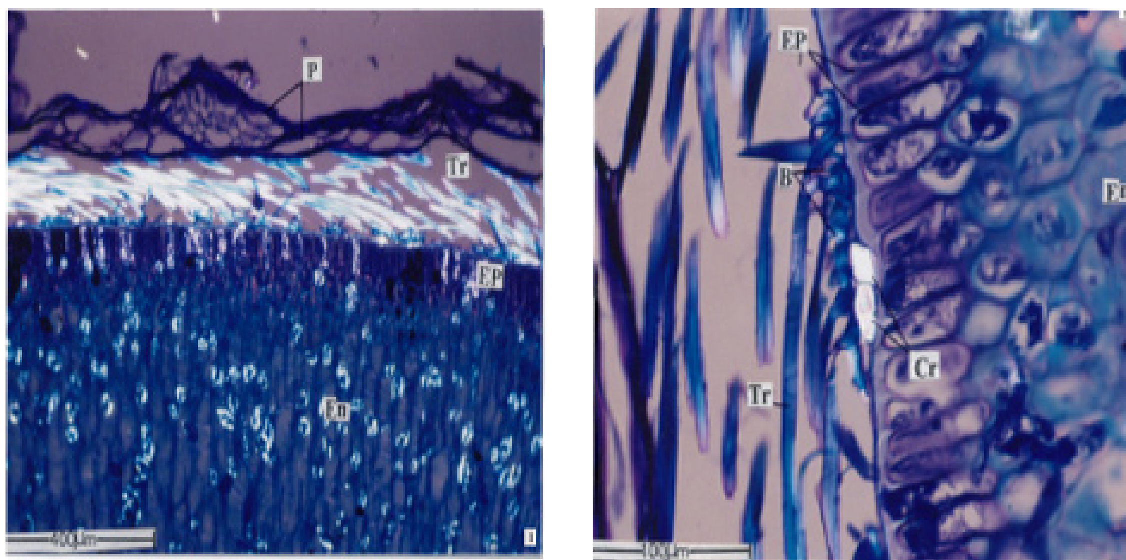


Figure 4 Seed coat under polarized light. 1. TS of the Testa and endosperm showing horizontally oriented trichomes. 2. Crystal deposition along the cuticle of the epidermis. B- Basal part of the Trichome ; Cr – Crystal ; En- Endosperm ; Tr- Trichome.

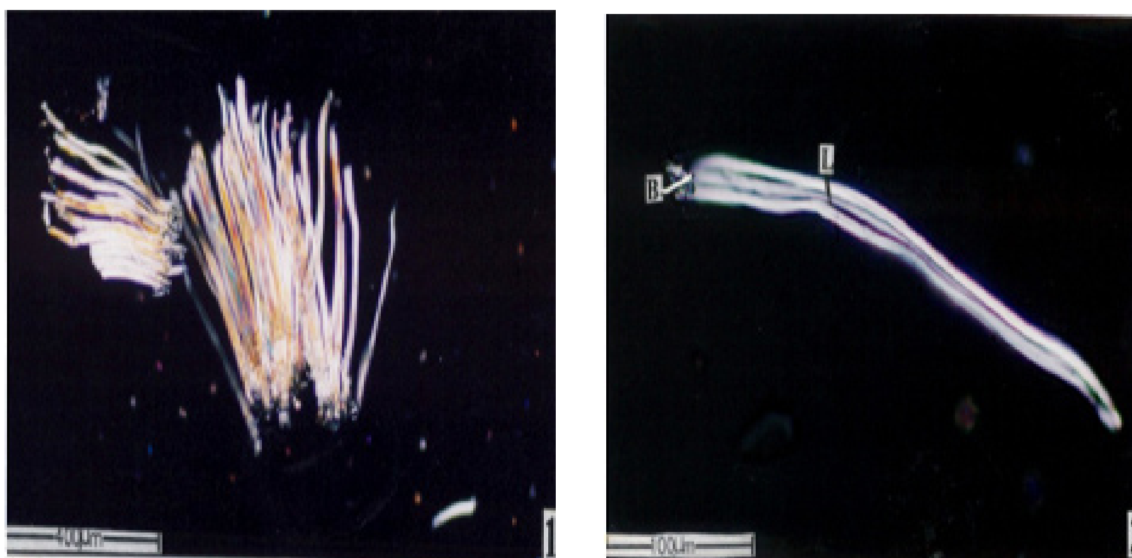


Figure 5 Trichomes of the seed under polarized light. 1. Bunch of trichomes scrapped out of the seed. 2. A single trichome showing the basal part cell lumen. B – Basal part of the trichome ; L – Lumen of the trichome.

of calcium oxalate crystals, by the details of structure of the trichomes and other features[21]. In transverse-vertical section, the seeds are elliptical and consist of a central cavity, which is broader in the middle and become gradually tapering on the opposite ends. The seed is occupied by massive endosperm around the central narrow elliptical cavity. The testa of the seed is uniformly thick in all parts of the seed.

Testa (seed coat)

The seed coat or testa consists of two distinct zones: the outer zone is somewhat uneven and consists of tangentially elongated, thin walled very much shrunken parenchyma, inner to the parenchyma zone is the trichome zone where dense, thick walled trichomes occur very close to each other. These trichomes have dilated basal part with which

the hairs are attached on the surface. The major portion of the trichome has thin narrow longitudinally running lumen. The unique feature of the trichomes is that all of them are bent at the base right angles and lie prostrate over the seed. The hairs are highly thick walled and walls are lignified. The lignification of the walls is evident, as the hairs appear brightly glittering when seen under the polarized light. It was also observed that calcium oxalate prismatic crystals were frequently seen on the surface of the seed i.e. at the basal part of the trichomes and on the surface of the endosperm tissue.

Endosperm

The endosperm tissue consists of vertically elongated, palisade-like epidermal cells on the surface zone of the seed. A thick and prominent cuticle is seen on the surface of the epidermis where the crystals occur. The cells inner to the palisade-like epidermal cells become gradually polygonal in outline. These cells have very thick walls and narrow lumen where cell inclusions are seen. The cell wall consists of cellulose and no lignification of the thick walls is evident. The narrow lumen of the endosperm cells contains nucleus and storage food materials. Plasmodesmatic connections are frequently seen crossing the cell walls and connecting the cytoplasm of adjacent cells.

Physiochemical constants

The physiochemical constants were determined and shown in table 1. The determination of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter, it is more especially applicable to powdered drugs. The total ash value is useful to exclude drugs, which have been coated with chalk, lime, or calcium sulphate to improve their appearance. The total ash value vary within wide limits for different specimens, whereas acid insoluble ash i.e. the ash insoluble in dilute hydrochloric acid, is often of more value than the total ash. The water-soluble ash is used to detect the presence of material exhausted by water. The water soluble ash is subjected to much greater reduction than is the total ash and is therefore used as an important indication of the presence of exhausted material substituted for the genuine article[21]. Alcohol soluble extractive value is frequently employed to determine the approximate resin content of the drug.

Determination of moisture content indicates the percentage of active chemical constituents in crude drugs mentioned on air-dried basis. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination[19].

Table 1: Physiochemical constants of *Strychnos potatorum* seed powder.

Moisture content	7.65%
Alcohol soluble extractive	4.39%
Water soluble extractive	12.25%
Total ash	1.43%
Acid insoluble ash	0.09%
Water soluble ash	–

Phytochemical screening

On detailed phytochemical screening by qualitative analysis, the SPP fractions showed the presence of steroids, triterpenoids, saponins and alkaloids in Phase I whereas flavonoids, tannins, carbohydrates, reducing sugars in Phase II. (Table 2)

Table 2: Phytochemical screening of SPP and SPE fractions by preliminary qualitative chemical analysis

Phytochemicals	SPP fractions					Methanol fraction of SPE (6)
	1	2	3	4	5	
Carbohydrates (Molisch's test)	–	–	–	+	+	+
Proteins and aminoacids (Millon's and Biuret test)	–	–	–	–	–	–
Alkaloids (Mayer's, Wagner's and Dragendorff's tests)	–	–	+	–	–	–
Steroids/Triterpenes (Libermann burchardt test)	+	+	–	–	–	+
Polyphenolics (Flavonoids/Tannins) Ferric chloride and Lead acetate test	–	–	–	+	+	+
Reducing sugars (Benedict's and Fehling's test)	–	–	–	+	+	+
Saponins (Foam test)	–	–	–	–	+	+

(+) Presence
(-) Absence.

HPTLC analysis

HPTLC profile of all the fractions showed well-resolved peaks on densitometric scanning at both UV light and after spraying specific reagents.

- SPP (Ether fraction): Scanning at 550 nm showed seven well resolved peaks, which were detected by spraying Vanillin sulphuric acid reagent. The Rf values were 0.09, 0.17, 0.26, 0.36, 0.45, 0.58 and 0.69 (Spectrum 1).
- SPP (Unsaponifiable fraction): At UV 260 nm, densitometric scanning gave five resolved peaks. The Rf values were 0.35, 0.43, 0.49, 0.60 and 0.71 (Spectrum 2).
- SPP (Alkaloid fraction): Scanning at UV 260 nm gave eight different peaks with Rf values 0.15, 0.21, 0.31, 0.39, 0.42, 0.54, 0.64 and 0.74 (Spectrum 3).

In conclusion, the pharmacognostic evaluation proves the authenticity of the seeds of *Strychnos potatorum* Linn. The preliminary chemical and chromatographic analysis of SPP and SPE showed the presence of phytochemicals like steroids, triterpenoids, saponins, polysaccharides and polyphenolics which may contribute various pharmacological activities of these drugs (SPP and SPE).

REFERENCES

1. Kirtikar KR, Basu BD. *Indian medicinal plants*. Allahabad; L.M. Basu, Vol **3**: p.1647; 1933.
2. Asima C, Satyesh CP. *The Treatise on Indian Medicinal Plants*. Publications and Information Directorate, New Delhi; CSIR, Vol **4**: p. 85–87; 2001.
3. Anonymous. *Wealth of India*. *Raw Materials*. *Sp-W*. Publications and Information Directorate, New Delhi; CSIR, Vol **10**: p. 66–67; 1976.
4. Harkishan Singh, Vijay K Kapoor, Phillipson JD, Bisset NG. Diaboline from Strychnos potatorum. *Phytochemistry*, **14**: 587–588, 1975.
5. Singh A, Bajpai RK. Chemical examination of fixed oil from the seeds of Strychnos potatorum Linn F. Mixed Fatty Acids. *Journal of Indian Chemical Society*, **3**: 768–769, 1975.
6. Singh AK, Dhar DN. Studies on the Chemical Constituents of the seeds of Strychnos potatorum L. Part II. *Planta Medica*, **32**: 362–367, 1977.
7. Harkishan Singh, Vijay K Kapoor. Investigation of Strychnos Spp.III Study of triterpenes and sterols of Strychnos potatorum seeds. *Planta Medica*, **28**: 392–396, 1975.
8. Venkata Rao E, Ramana KS, Venkateswarao M. Revised structure and antihypercholesterolemic activity of a mannogalactan from Strychnos potatorum. *Indian Journal of Pharmaceutical Sciences*, **53**(2): 53–57, 1991.
9. Corsaro MM, Giuddiciani I, Lanzetta R, Marciano CE, Monaco P and Parilli M. Polysaccharides from seeds of Strycnos species. *Phytochemistry*, **39**(6): 1377–1380, 1995.
10. Adinolfi M, Corsaro MM, Lanzetta R, Parilli M, Folkard G, Grant W, Sutherland J. Composition of the coagulant polysaccharide fraction from Strychnos potatorum. *Carbohydrate Research*, **263**: 103–110, 1994.
11. Harkishan Singh, Vijay K Kapoor. Investigation of Strychnos Spp. V1.Pharmacological studies of alkaloids of Strychnos potatorum seeds. *Planta Medica*, **38**: 133–137, 1980.
12. Harkishan Singh, Kapoor VK. Investigation of Strychnos species. IV. Pharmacological studies of alkaloids of Strychnos potatorum. *Planta Medica*, **29**: 226–233, 1976.
13. Swathi B, Murugesan T, Sanghamitra Sinha et al. Antidiarrhoeal activity of Strychnos potatorum seed extract in rats. *Fitoterapia* **73**: 43–47, 2002.
14. Biswas S, Murugesan T, Maiti K, Ghosh Let al. Study on the diuretic activity of Strychnos potatorum Linn, seed extract in albino rats. *Phytomedicine*, **8** (6): 469–471, 2001.
15. Mathuram LN, Samanna HC, Ramasamy VM, Natarajan R. Studies on the hypoglycemic effects of Strychnos potatorum and Acacia arabica on alloxan diabetes in rabbits. *Cheiron*, **10** (1): 1–5, 1981.
16. Sass JE, Elements of botanical microtechnique, Newyork; McGraw Hill, p.222, 1940.
17. O'Brien TP, Feder N, McCull ME. Polychromatic staining of plant cell wall by toluidine blue-O. *Protoplasma*. **59**: 364–373, 1964.
18. *Ayurvedic Pharmacopoeia of India*. *Part-1*, New Delhi, Controller of Publications, Vol **II**: p. 190–191, 1999.
19. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*, 5th Ed., India, Nirali Prakashan Publications, p.109–137, 1997.
20. Trease GE, Evans WC. *Pharmacognosy*, 12th ed., London, Balliere-Tindall, p.241–260, 1983.
21. Wallis TE. *Textbook of Pharmacognosy*, 5th ed., New Delhi; BS Publishers and Distributors, p. 558, 1985.

Phytochemical profile of selected Philippine plants used to treat asthma

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ABSTRACT

Phytochemical analyses were carried out to determine the similarities of the plant metabolites present in the various plants used traditionally for the alleviation of asthma. Plants were collected from the University of the Philippines Diliman campus. The dried samples were homogenized for overnight soaking in methanol at room temperature. The resulting alcoholic extracts were filtered and concentrated *in vacuo* and tested for their various metabolites. Saponins, terpenoids, flavonoids and cardiac glycosides were commonly found in the various plant samples and to some extent, the anti-asthma activity of the plants could be attributed to these types of secondary metabolites.

Keywords: *asthma, plant extracts, phytochemical.*

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INTRODUCTION

Asthma is a national health priority because of its increasing prevalence and significant impact on the quality of life.[1, 2] The cost of severe asthma is similar to cardiovascular diseases [3] and has been associated with depression.[4, 5] It is estimated that 300 million people worldwide are suffering from this condition.[6] Around 8 million Filipinos are suffering from asthma according to the International Study of Asthma and Allergies in Childhood and the prevalence of the condition is increasing especially in children. Various medications are available, however, the relief they offer is mainly symptomatic and short-lived, on top of their unwanted side-effects.[7–10] Hence, there is a need for safer and effective medications for asthma. Screening of plants used in traditional medicine is a potential source of compounds that could be developed into more effective asthma drugs.

Thorough literature reviews of plants used in Philippine herbal medicine have shown several plants that are use for the treatment of asthma. Quisumbing [11] has reported several such plants and this includes *Bambusa blumeana* Schultes ('kauayan'), *Pistia stratiotes* Linn ('kiapo'), *Isotoma longiflora* ('estrella'), *Monochoria vaginalis* ('gabing-uak'), *Plumeria rubra* ('kalachuche'), *Euphorbia hirta* ('gatas-gatas'), *Amaranthus viridis* ('kolitis'), *Commelina diffusa* ('alikbangon') and *Eclipta alba* ('tinta-tinta'). *B. blumeana* is also used as a stimulant, astringent, antispasmodic and for coughs. [11] The leaves of *P. stratiotes* is mixed with sugar and used for coughs and asthma.[11] It is used to treat gastroenteritis and fever in Africa.[12] Its interaction with several metals has been studied [13–16] and its possible phytoremediation activity.[17–19] The antiviral, [20] anti-degerative effect, [21] anti-anaphylactic, [22] antimicrobial, [23] anti-allergy, [24] and anti-diarrheal [25] action of *E. hirta* have been investigated. Butanol rhamnosides have also been isolated from its polar and non-polar extracts.[26] *E. alba* has shown hair-growth promoting activity, [27, 28] anti-venom, [29] anti-malarial, [30] anti-aggressive, [31] and anti-hypertensive action.[32] *I. longiflora* is used for its antibacterial properties in Puerto Rico.[33] The leaves of *M. vaginalis* are used for diabetes in India (ext-link-type="uri" xmlns:xlink="http://www.w3.org/1999/xlink" xlink:href="http://www.eseap.cipotato.org">http://www.eseap.cipotato.org). *A viridis* is eaten as a vegetable in India and used as a medicinal herb in Ayuverdic medicine.[34] *C. diffusa* is used as

febrifuge and treatment for diabetes in China.[35] *P. rubra* is a traditional medicinal plant used in Thailand for its molluscicidal and antibacterial activity.[36] It is also one of the five flowers used in China as an herbal beverage for sore throat, halitosis, constipation and irritability.[37]

Different activities have been attributed to these plants. However, no study has been previously reported on the phytochemical constituents of these plants. Therefore, this research aims to determine the phytochemical profile of these plants and establish whether the presence of certain metabolites is responsible for its traditional use.

MATERIAL AND METHOD

Plant material

Leaves were collected from the University of the Philippines, Diliman Campus, Quezon City. A voucher specimen for each plant was submitted to the Dr. Jose

Table 1: Phytochemical tests

Test	Procedure	Expected Result
Tannins	Dissolve around 2 mg of sample in 5 mL distilled water. Add 15° $FeCl_3$ solution drop wise.	The formation of a brownish-green precipitate indicates condensed tannins while the appearance of a blue-black precipitate indicates the presence of hydrolysable tannins.
Saponins	Boil approximately 5 mg of sample in 5 ml distilled water. Allow the mixture to cool then vigorously shake.	Frothing suggests the presence of saponins.
	Another test is to layer the solution with corn oil then shake vigorously.	Presence of emulsion at the froth-water interface.
Terpenoids	Prepare a TLC chromatogram of the samples using Silica Gel 60 F_{254}. Develop the chromatogram in $CHCl_3$ then spray with vanillin-H_2SO_4 solution.	The formation of red to purple spots upon heating of the TLC plate.
	Confirmatory analysis is done using the Salkowski Test. Treat 2 mg of the sample with 2 ml $CHCl_3$ then layer with H_2SO_4	Formation of reddish-brown color at the interface.
Flavonoids	Dissolve around 2 mg of the sample in 2 ml of 1M NaOH followed by the addition of several drops of 0.6M HCl	A yellow to orange solution with NaOH that turns colorless upon addition of HCl.
	Confirmatory test is done by dissolving the sample in 70° EtOH then spot on a Silica Gel 60 TLC plate. Develop the chromatogram in Forestal solution (30:3:10 glacial acetic acid: concentrated HCl: water), allow to dry, then view under UV light, and then fume with NH_4OH.	Note for fluorescence or change in color.
Cardiac glycosides	Killiani-Keller test — add one drop of 15° $FeCl_3$ to 2 mg of sample dissolved in 2 ml distilled water. Then layer the solution in 1 ml concentrated H_2SO_4.	Formation of a brown ring at the interface indicates the presence of cardiac glycosides.
Phenolic compounds	Add a few drops of 1° $FeCl_3$ to two mg of sample dissolved in 2 ml distilled water.	Formation of a green, purple, blue, or black solution.
Steroids	Add 2 ml of diluted H_2SO_4 to 2 mg of sample dissolved in 2 ml acetic anhydride.	Formation of a blue or green solution.
Alkaloids	Dissolve 5 mg of sample in 2 mL distilled water then add 3 drops of Wagner's reagent. This reagent is prepared from 2 g iodine and 6 g potassium iodide dissolved in 100 mL water.	Formation of a blue black precipitate.

Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman.

Plant extraction

Dried leaves were air-dried, homogenized, weighed then soaked in methanol (MeOH). The resulting MeOH extracts were concentrated *in vacuo* at 40°C using a rotary evaporator. The MeOH used was technical grade and distilled before use.

Phytochemical analysis

Phytochemical screening of the crude extracts was based on several procedures with slight modifications [38–40] as shown in Table 1.

RESULTS AND DISCUSSION

The phytochemical profiles of nine Philippine plants that are being used in traditional medicine have been determined. Phytochemical screening of the different plants revealed that they contain several types of metabolites as shown in Table 2. It is worth noting that most of the extracts were positive for saponins, terpenoids, flavonoids and cardiac glycosides. It is probable that such compounds that were found to be present in the plants are responsible for their anti-asthma activity.

Several studies have shown that compounds from these classes inhibit asthma. For instance, astragaloside IV, a new cycloartane-type triterpene glycoside extract of *Astragalus membranaceus* Bunge, has been shown to inhibit ovalbumin-induced chronic experimental asthma.[41] Triterpenoid saponins have been identified from the stem bark of *Pteleopsis suberosa*, a traditional remedy for asthma.[42] The seeds of *Allium tuberosum* which is also used for asthma have yielded steroid saponins.[43] A triterpenoid glycoside isolated from *Bupleurum falcatum* has shown inhibitory action against allergic asthma in rats.[44] Flavonoids found in saboku-to, a herbal medicine for bronchial asthma, have shown inhibitory action against the release of leukotrienes from human leukocytes.[45[Licorice flavonoids inhibit airway eonosiphilic inflammation which is a major feature of allergic asthma.[46]

CONCLUSIONS

To some extent, the observed inhibition of asthma could be attributed to the various plant secondary metabolites detected in the plant materials. Further studies are currently being done to determine which plant exhibits the highest activity against asthma.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTION

CCH designed the study and was involved in the preparation of the manuscript while NDP was involved in the experiments and collection of data.

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Table 2: Phytochemical profile of the nine Philippine plants

Sample	Tannin	Saponin	Terpenoid	Flavonoid	Cardiac Glycoside	Phenolic Compounds	Steroids	Alkaloid
1. B. spinosa	–	–	+	–	–	–	–	–
2.P.stratiotes	–	+	+	+	–	–	–	–
3. I. longiflora	–	+	–	–	+	–	–	–
4. M. vaginalis	–	+	+	+	–	–	–	–
5. P. rubra	–	+	+	–	+	–	–	–
6. E. hirta	+ blue black ppt.	+	+	+	+	+	+	–
7.A. viridis	–	+	–	–	+	–	–	–
8. C. diffusa	–	+	+	+	+	+	–	–
9. E. alba	+ brownish green ppt.	–	+	+	+	+	–	–

REFERENCES

1. Adams R, Wakefield M. Quality of life in asthma: a comparison of community and hospital asthma patients. *Journal of Asthma*, **38**: 205–214, 2001.
2. Manocchia M, Keller S, Ware JE. Sleep problems, health-related quality of life, work functioning and health care utilisation among the chronically ill. *Quality of Life Research*, **10**: 331–345, 2001.
3. Serra-Batlles J, Plaza V, Morejun E, Cornelia A, Brugues: Costs of asthma according to degree of severity. *European Respiratory Journal*, **12**: 1322–1326, 1998.
4. Mancuso CA, Rincon M, McCulloch CE, Charlson ME. Self-efficacy, depressive symptoms, and patients' expectation predict outcomes in asthma. *Medical Care*, **39**: 1326–1338, 2001.
5. Rimington LD, Davies DH, Lowe D, Pearson MG. Relationship betweeen anxiety, depression, and morbidity in adult asthma patients. *Thorax*, **56**: 266–271, 2001.
6. Dahl R. Systemic side-effects of inhaled corticosteroids in patients with asthma. *Respiratory Medicine*, **100**: 1301–1317, 2006.
7. Barnes P. Current issues for establishing inhaled corticosteroids as the anti-inflammatory agents of choice in asthma. *Journal of Allergy and Clinical Immunology*, **101**: S427–S433, 1998.
8. Ihre E, Zetterstrom O, Hammarberg. Voice problems as side effects of inhaled corticosteroids in asthma patients – a prevalence study. *Journal of Voice*, **18**: 403–414, 2004.
9. Kos-Kudla B, Pluskiewicz W. Quantitative ultrasound of the heel and serum and urinary cortisol values in assessment of long-term corticotherapy of long-term side effects in female bronchial asthma patients. *Ultrasound in Medicine and Biology*, **23**: 1325–1330, 1997.
10. White MV, Sander N. Asthma from the perspective of the patient. *Journal of Allergy and Clinical Immunology*, **104**: S47–S52, 1999.
11. Quisumbing E. *Medicinal plants from the Philippines*. Katha Publishing, Quezon City, Philippines; 1978.
12. Tabuti JRS, Dhillion SS, Lye KA. Ethnoveterinary medicines for cattle in Bulamogi county, Uganda: plant species and mode of use. *Journal of Ethnopharmacology*, **88**: 279–286, 2003.
13. Odjegba VJ, Fasidi IO. Changes in antioxidant enzyme activities in Eichhornia crassipes (Pontederiaceae) and Pistia stratiotes (Araceae) under heavy metal stress. *Revista de Biologia Tropical*, **55**: 815–823, 2007.
14. Sinha S, Saxena R, Singh S. Chromium induced lipid peroxidation in the plants of Pistia stratiotes L.: role of antioxidants and antioxidant enzymes. *Chemosphere*, **58**: 595–604, 2005.
15. Sinha S, Basanta A, Malik A, Singh K. Multivariate modeling of chromium-induced oxidative stress and biochemical changes in plants of Pistia stratiotes. *Ecotoxicology*, **18**: 555–566, 2009.
16. Upadhyay RK, Panda SK. Copper-induced growth inhibition oxidative stress and ultrastructural alterations in freshly grown water lettuce (Pistia stratiotes). *Comptes rendus biologies*, **332**: 623–632, 2009.
17. Gujarathi NP, Haney BJ, Linden JC. Phytoremediation potential of Myriophyllum aquaticum and Pistia stratiotes to modify antibiotic growth promoters, tetracycline and oxytetracycline in aqueous wastewater systems. *International Journal of Phytoremediation*, **7**: 99–112, 2005.
18. Odjegba VJ, Fasidi IO. Accumulation of trace elements by Pistia stratiotes: implications of phytoremediation. *Ecotoxicology*, **13**: 637–646, 2004.
19. Tewari A, Singh R, Singh NK, Rai UN. Amelioration of municipal sludge by Pistia stratiotes L.: role of antioxidant enzymes in detoxification of metals. *Bioresource Technology*, **99**: 8715–8721, 2008.
20. Gyuris A, Szlavik L, Minarovits J, Vasas A, Molnar J, Hohmann J. Antiviral activities of extracts of Euphorbia hirta L against HIV-1, HIV-2 and SIVmac251. *In vivo*, **23**: 429–432, 2009.
21. Lee KH, Chen YS, Judson JP, Chakravarthi S, Sim YM, Er HM. The effects of water extracts of Euphorbia hirta on cartilage degeneration in arthritic mice. *Malaysian Journal of Pathology*, **30**: 95–102, 2008.
22. Youssouf MS, Kaiser P, Tahir M, Singh GD, Singh S, Sharma VK, et al. Anti-anaphylactic effect of Euphorbia hirta. *Fitoterapia*, **78**: 535–539, 2007.
23. Sudhakar M, Rao CV, Rao PM, Raju DB, Ventekateswarlu Y. Antimicrobial activity of Caesalpinia pulcherrima, Euphorbia hirta and Asystasia gangeticum. *Fitoterapia*, **77**: 378–380, 2006.
24. Singh GD, Kaiser P, Youssouf MS, Singh S, Kharjuria A, Koul A, et al. Inhibition of early and late phase allergic reactions by Euphorbia hirta L. *Phytotherapy Research: PTR*, **20**: 316–321, 2006.
25. Hore SK, Ahuja V, Mehta G, Kumar P, Pandey SK, Ahmad AH. Effect of Euphorbia hirta leaf extracts on gastrointestinal motility. *Fitoterapia*, **77**: 35–38, 2006.
26. Mallavadhani UV, Nasasimhan K. Two novel butanol rhamnosides from an Indian traditional herb, Euphorbia hirta. *Natural Product Research*, **23**: 644–651, 2009.
27. Datta K, Singh AT, Mukherjee A, Bhat B, Ramesh B, Burman AC. Eclipta alba extract with potential for hair growth promoting activity. *Journal of Ethnopharmacology*, **124**: 450–456, 2009.
28. Roy RK, Thakur M, Dixit VK. Hair growth promoting activity of Eclipta alba in male Albino rats. *Archives of Dermatological Research*, **300**: 357–364, 2008.
29. Diogo LC, Fernandez RS, Marcussi S, Menaldo DL, Roberto PG, Mantrangulo PVF, et al. Inhibition of snake venoms and phospholipases A_2 by extracts from native and genetically modified Eclipta alba: isolation of active coumestans. *Basic Clinical Pharmacology and Toxicology*, **104**: 293–299, 2009.
30. Bapna S, Adsule S, Shirshat MS, Jadhav S, Patil LS, Deshmukh RA. Anti-malarial activity of Eclipta alba against Plarmdocium berghei infection in mice. *The Journal of Communicable Diseases*, **39**: 91–94, 2007.
31. Lobo OTJ, Banji D, Annamalai AR, Manavalan R. Evaluation of anti aggressive activity of Eclipta alba in experimental animals. *Pakistan Journal of Pharmaceutical Science*, **21**: 195–199, 2008.
32. Rangineni V, Sharada D, Saxena S. Diuretic, hypotensive and hypocholesterolemic effects of Eclipta alba in mild hypertensive subjects: a pilot study. *Journal of Medicinal Food*, **10**: 143–148, 2007.
33. Melendez P, Capriles V. Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine*, **13**: 272–276, 2006.
34. Nair RV. *Controversial drug plants*. India: Universities Press Private Limited; p. 270, 2004.
35. Hong D, Defillips RA. *Commelina diffusa Flora of China*. Beijing Science Press; p. 36, 2000.
36. Hamburger MO, Cordell GA, Ruangrungsi N. Traditional medicinal plants of Thailand. XVII. Biologically active constituents of Plumeria rubra. *Journal of Ethnopharmacology*, **33**: 289–292, 1991.
37. Kong FY, Ng DK, Chan CH, Yu WL, Chan D, Kwok KL, et al. Parental use of the term 'hot-qi' to describe symptoms in their children in Hong Kong: a cross sectional survey 'hot-qi' in children. *Journal of Ethnobiology and Ethnomedicine*, **2**: 2–8, 2006.
38. Edeoga HO, Okwu DE. Phytochemical Constituents of Some Nigerian Medicinal Plants. *African Journal of Biotechnology*, **4**: 685–688, 2005.
39. Harborne JB. *Phytochemical Methods*. A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, USA; p. 298, 1984.
40. Onwukaeme DN, Ikuegbvwehaand TB, Asonye CC. Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of Pycanthus Angolensis Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits. *Tropical Journal of Pharmaceutical Research*, **6**: 725–730, 2007.
41. Du Q, Chen Z, Zhou LF, Zhang Q, Huang M, Yin KS. Inhibitory effects of astragaloside IV on ovalbumin-induced chronic experimental asthma. *Canadian Journal of Physiology and Pharmacology*, **86**: 449–457, 2008.

42. Leo MD, Tommasi ND, Sanogo R, D'Angelo V, Germano MP, Bisignano O G, Braca A. Triterpenoids saponins from Pteleopsis suberosa stem bark. *Phytochemistry*, **67**: 2623–2629, 2006.
43. Sang S, Mao S, Lao A, Chen Z, Ho CT. New steroid saponins from the seeds of Allium tuberosum L. *Food Chemistry*, **83**: 499–506, 2003.
44. Park KH, Park J, Koh D, Lim Y. Effect of saikosaponin-A, a triterpenoid glycoside isolated from Bupleurum falcatum on experimental allergic asthma. *Phytotherapy Research: PTR*, **16**: 359–363, 2002.
45. Homma M, Minami M, Taniguchi C, Oka K, Morita S, Niitsuma T, et al. Inhibitory effects of lignans and flavonoids in saiboku-to, a herbal medicine for bronchial asthma, on the release of leukotrienes from human polymorphonuclear leukocytes. *Planta Medicine*, **66**: 88–91, 2002.
46. Jayaprakasam B, Doddaga S, Wang R, Holmes D, Goldfarb J, Li XM. Licorice flavonoids inhibit eotaxin-1 secretion by human fetal lung fibroblasts in vitro. *Journal of Agricultural and Food Chemistry*, **57**: 820–825, 2009.

Antibacterial activities of various sequential extracts of *Ficus racemosa* stem bark

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Abstract

The present study evaluated the antibacterial activity of sequential extracts of *Ficus racemosa* stem bark against *Staphylococcus aureus* [MTCC 3160], *Bacillus cereus* [MTCC 1306], *Pseudomonas aeruginosa* [MTCC 1034], *Escherichia coli* [MTCC 1089] and *Bacillus subtilis* [MTCC 1133] by disk-diffusion and agar-diffusion methods. In disk-diffusion assay chloroform, acetone and methanol extracts showed moderate antibacterial against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* compared to the positive control, while petroleum ether extract did not exhibit antibacterial activity against any of the organisms tested. Aqueous extract inhibited only *Bacillus subtilis*, while none of the extracts inhibited *Pseudomonas aeruginosa*. In agar-diffusion assay, both petroleum ether and aqueous extract did not show any inhibitory activity against any of the test organisms, while methanol extract showed moderate activity against *Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli*. Acetone extract showed moderate inhibition of *Staphylococcus aureus, Bacillus cereus* and FRSCE inhibited *Bacillus subtilis* and *Escherichia coli* to some extent.

Keywords: *Ficus racemosa*, Antibacterial, Sequential extracts, Moraceae, MIC.
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INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance.[1] Research on new antimicrobial substances from natural products must therefore be continued. Current research on natural molecules and products primarily focuses on plants since they can be sourced more easily and be selected on the basis of their ethnomedicinal use.[2]

Different parts of *F. racemosa* have been shown to possess significant antibacterial activity. Mandal et al. evaluated various extracts of *F. racemosa* leaves for antibacterial potential against *Escherichia coli*, *Basillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was found that the petroleum ether extract was most effective against the tested organisms and the effect produced was significant and was compared with chloramphenicol, a known antibiotic.[3] The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of *F. racemosa* effectively inhibited the growth of *Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp, Corynespora cassiicola* and *Fusarium sp*.[4]

With this background, the present study evaluated the antibacterial activity of the sequential extracts of *F. racemosa* bark against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* using disk-diffusion and agar-diffusion assay.

MATERIALS AND METHODS

Chemicals and plant material

Ficus racemosa stem bark was collected from Mukkadahally, Chamarajanagar district of Karnataka, India during September 2007, subsequently identified by Dr. Shivprasad Huded, JSS Ayurvedic Medical College, Mysore, and the voucher specimen [BOT-001/2008] was deposited at the

herbarium of Department of Studies in Botany, University of Mysore, Mysore, India. The bark was cut into small pieces, dried [50°C] and powdered, passed through 60 mesh sieve [BS] and stored in an air tight container at 4°C till further use. All the other reagents and chemicals used in the study were of extra pure analytical grade.

Micro-organisms

The five bacterial strains of Microbial Type Culture Collection [MTCC] namely, *Staphylococcus aureus* [MTCC 3160], *Bacillus cereus* [MTCC 1306], *Pseudomonas aeruginosa* [MTCC 1034], *Escherichia coli* [MTCC 1089] and *Bacillus subtilis* [MTCC 1133] were obtained from the Institute of Microbial Technology [IMTECH], Chandigarh, India.

Preparation of extracts

The bark powder [100 g] was extracted sequentially with petroleum ether, chloroform, acetone, methanol and water in a soxhlet extractor by continuous hot percolation to yield sequential petroleum ether extract [FRSPE], sequential chloroform extract [FRSCE], sequential acetone extract [FRSACE], sequential methanol extract [FRSME] and sequential aqueous extract [FRSAE]. Each time before extracting with the next solvent of higher polarity the powdered drug was dried in a hot air oven below 50°C for 10 min. solvents were evaporated in a rotary vacuum evaporator and the dried extracts were weighed [Bhattacharya & Zaman, 2009].

Antibacterial activity

Antibacterial activity of various sequential extracts of *F. racemosa* bark was evaluated by disk-diffusion method employing 24 h cultures of five test organisms including two Gram-positive bacteria; *Staphylococcus aureus* and *Bacillus cereus*, three Gram-negative bacteria; *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. The test organisms were inoculated into sterile nutrient agar medium by uniformly mixing 1 mL of inoculum with 20 mL sterile melted nutrient agar cooled to 48°–50°C, in a sterile Petri dish. When the agar solidified, eight holes of uniform diameter [6 mm] were made by using a sterile borer. Three volumes of each of the test solutions as well as standard solution [Chloramphenicol] and the blank [respective solvents] were placed in each hole separately under specific condition and the plates were then maintained at room temperature for 2 h to allow the diffusion of the solution into the medium. All the plates were then incubated at 37°C for 24 h and the zone of inhibition was measured.[5]

Determination of minimum inhibitory concentration [MIC]

Minimum inhibitory concentrations of various sequential extracts were determined using agar dilution assay.[5] A loopful of the bacterial culture from the slant is inoculated in the nutrient broth and incubated at 37°C for 24 h. The fresh broth [20 mL] is seeded with 0.25 mL of the 24 h broth cultures and two fold serial dilution method is followed as described below. The test sample was dissolved in water or solvent to obtain 10mg/mL solution. A 0.2 mL of the solution of test material is added to 1.8 mL of the seeded broth and this form the first dilution.

1 mL of this dilution is diluted further with 1 mL of the seeded broth to produce the second dilution, and the process is repeated until six such dilutions are obtained. A set of tubers containing only seeded broth is kept as control and suitable solvent controls are also maintained. After incubation for 24 h at 37°C the last tube with no visible growth of the microorganism is taken to represent the minimum inhibitory concentration of the test sample which is expressed in μg mL^{-1}.

RESULTS

The antibacterial activities of different extracts were indicated by the zone of inhibition [Table 1]. In disk diffusion assay FRSCE, FRSACE and FRSME showed moderate antibacterial against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* compared to the positive control. FRSPE did not exhibit antibacterial activity against any of the organisms tested while, FRAE inhibited only *Bacillus subtilis*. None of the extracts exhibited antibacterial activity against *Pseudomonas aeruginosa*.

In agar-diffusion assay, both FRSPE and FRSAE did not show any inhibitory activity against any of the test organisms, while, FRSME showed moderate activity against *Staphylococcus aureus, Bacillus subtilis,* and *Escherichia coli*. FRSACE inhibited *Staphylococcus aureus, Bacillus cereus* and FRSCE inhibited *Bacillus subtilis* and *Escherichia coli* to some extent [Table 2].

The minimum inhibitory concentrations of various extracts for different bacteria are presented in Table 3. FRSCE and FRSME showed a MIC value of 100 μg mL^{-1} against *Escherichia coli*. FRSACE showed a value of 150 μg mL^{-1} for *Staphylococcus aureus* and *Bacillus cereus*, while FRSME showed a value of 150 μg mL^{-1} for *Staphylococcus aureus* and *Bacillus subtilis*.

DISCUSSION

Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics.

Table 1. Zone of inhibition [mm] for various micro-organisms

	Staphylococcus aureus	Bacillus cereus	Bacillus subtilis	Pseudomonas aeruginosa
FRSPE	-	-	-	-
FRSCE	6	6	6	-
FRSACE	6	6	6	-
FRSME	7	8	6	-
FRSAE	-	-	6	-
Control	28	30	28	15

* *Control: antibiotic disc containing 30 μg of Chloramphenical C30*

** *discs diameter 5 mm, each disc impregnated to contain 4 μl [1 mg mL^{-1}] of solution.*

Table 2. Zone of inhibition [mm] for various micro-organisms

	Staphylococcus aureus	Bacillus cereus	Bacillus subtilis	Pseudomonas aeruginosa	Escherichia coli
FRSPE	-	-	-	-	-
FRSCE	-	6	-	-	9
FRSACE	6	7	-	-	-
FRSME	6	-	8	-	6
FRSAE	-	-	-	-	-
Cephalexin	15	25	25	25	25

** *Each well filled with 50 μl [1 mg mL^{-1}] of solution.*

Table 3. Minimum inhibitory concentrations of different *Ficus racemosa* bark extracts against various micro-organisms

	Staphylococcus aureus	Bacillus cereus	Bacillus subtilis	Pseudomonas aeruginosa	Escherichia coli
FRSPE	-	-	-	-	-
FRSCE	-	150	-	-	100
FRSACE	150	150	-	-	-
FRSME	150	-	150	-	100
FRSAE	-	-	-	-	-

** *Each well filled with 50 μL[1 mg mL^{-1}] of solution.*

In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives.[6] Various solvent extracts from *F. racemosa* bark showed moderate inhibiting activity on disease causing Gram-negative and Gram-positive bacteria, the most inhibited being *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis,* and *Escherichia coli*. This is particularly interesting from a medical point of view because these microbial agents are responsible for severe opportunistic infections.[7] Our findings on antibacterial activity of *F. racemosa* bark could justify some ethnopharmacological uses such as against diarrhea and dysentery because we demonstrated good activity of this plant against some pathogens of the digestive tract. The antimicrobial activity of FRSACE, FRAME and FRSAE could be attributed to the presence of phenolic substances which is well documented.[8]

These findings are in good agreement with a number of earlier studies, wherein antibacterial potential of *F. racemosa* against different bacterial strains are reported. Nair and Chanda reported that the ethanol extract of the stem bark was effective against *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Bacillus cereus, Alcaligenes faecalis* and *Salmonella typhimurium* bacterial strains, indicating the scope to discover bioactive natural

products that may serve as leads in the development of new pharmaceuticals in order to address unmet therapeutic needs.[9] In another study the same authors reported that the ethanol extract of stem bark exhibited significant antibacterial activity against *Pseudomonas aeruginosa, Proteus mirabilis and Bacillus cereus* bacterial strains while the aqueous extract inhibited *Streptococcus fecalis* significantly[10] and the methanol extract exhibited significant antibacterial activity against *Bacillus subtilis*.[11]

Other parts of *F. racemosa* tree have also shown significant antibacterial activity. Mandal et al. evaluated various extracts of *F. racemosa* leaves for antibacterial potential against *Escherichia coli, Basillus pumilis, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was found that the petroleum ether extract was most effective against the tested organisms and the effect produced was significant and was compared with chloramphenicol, a known antibiotic, supporting the use of *F. racemosa* for treating dysentery and diarrhea in the traditional system of medicine.[3] The 50% methylene chloride in hexane flash column fraction of the extract of the leaves of *F. racemosa* effectively inhibited the growth of *Curvularia sp, Colletotrichum gloeosporioides, Alternaria sp, Corynespora cassiicola* and *Fusarium sp*.[4]

The reported wound healing property of *F. racemosa* may also be due to its ability to inhibit the growth of micro-organisms thereby preventing infection and accelerating the process of wound healing. This view can be supported form a study, wherein the ointment prepared from the *F. racemosa* leaf powder in an 8 mm full-thickness punch wound rat model showed highly significant generation of tissue DNA, RNA, and total protein during healing process in comparison with untreated control rats.[12]

From the results of this investigation, it is inferred that *F. racemosa* stem bark possesses potential antibacterial activity against certain micro-organisms. However, the results are not highly encouraging for the development and utilization of *F. racemosa* bark extracts as antibiotics, as the antibacterial activity exhibited by them was moderate and not excellent/exceptional. But, it is of interest, rather useful to use *F. racemosa* bark in infections of gastrointestinal tract caused by the bacterial pathogens, as the anti-diarrheal and gastroprotective activity of the bark is very well established.

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REFERENCES

1. Okeke IN, Laxmaninarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP [2005], Antimicrobial resistance in developing countries. Part 1: recent trends and current status. *Lancet Infectious Diseases*, **5**:481¦493.
2. Verpoorte R, Choi YH, Kim HK [2005], Ethnopharmacology and systems biology: a perfect holistic match. *J Ethnopharmacol*, **100**:53¦56.
3. Mandal SC, Saha BP, Pal M [2000], Studies on antibacterial activity of *Ficus racemosa* Linn. leaf extract. *Phytother Res*, **14**:278¦280.
4. Deraniyagala SA, Wijesundera RLC, Weerasena OVDSJ [1998], Antifungal activity of Ficus racemosa leaf extract and isolation of the active compound. *J Natn Sci Coun Sri Lanka*, **26**:19¦26.
5. Atta-ur-Rahman, Choudhary MI, Thomsen WJ [2005], *Bioassay techniques for drug development*. Harwood Academic publishers, Australia.
6. Penso G [1980], The role of WHO in the selection and characterization of medicinal plants [vegetable drugs]. *J Ethnopharmacol*, **2**[2]:183¦188.
7. Basile A, Ferrara L, Pezzo MD, Mele G, Sorbo S, Bassi P, Montesano D [2005]:Antibacterial and antioxidant activities of ethanol extract from Paullinia cupana Mart. *J Ethnopharmacol*, **102**:32¦36.
8. Karamanoli K [2002], *Secondary metabolites as allelochemicals in plant defence against microorganisms of the phyllosphere*. Science Publishers Inc., NH, USA, pp. 277¦288.
9. Nair R, Chanda SV [2007], Antibacterial activities of some medicinal plants of the western region of India. *Turkish J Biol*, **31**:231¦236.
10. Nair R, Chanda SV [2006], Activity of some medicinal plants against certain pathogenic bacterial strains. *Indian J Pharm*, **38**:142¦144.
11. Mahato RB, Chaudhary RP [2005], Ethnomedicinal study and antibacterial activities of selected plants of Palpa district, Nepal. *Scientific World*, **3**:26¦31.
12. Biswas TK, Mukherjee B [2003], Plant medicines of Indian origin for wound healing activity: A Review. *Intl J Lower Extremity Wounds*, **2**:25¦39.

Acute toxicity studies of Some Indian Medicinal plants.

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ABSTRACT

It is presumed that ayurvedic drugs have lesser side effects as compared to allopathic drugs. For the safety to use these plants and preparations (gel and powder forms), the medicinal plants need to be evaluated for their toxicity. The aim of this study was to test the acute toxicity of three medicinal plants, *Terminalia bellerica* (Gertn.) Roxb. fruits, *Moringa pterigosperma* (Gaertn) leaves, *Cassia tora Linn.*leaves. The acute toxicity study was studied on Swiss mice with a dose of 3 and 5 g/Kg body weight orally. The single administration exposure of the whole plant powder in the form of aqueous slurry on Swiss mice was carried out and the exposure route was oral with water as a vehicle. The observations of changes in body weight, food and water intake as well as cage side observations were reported. The plants were found to be nontoxic

Keywords: *Terminalia bellerica*, *Moringa pterigosperma*, *Cassia tora*, acute toxicity.

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INTRODUCTION

Toxicity is the fundamental science of poisons. The organization for Economic and Development (OECD) mentioned acute toxicity as the advance effect occurring within a short time of oral administration of a simple dose of a substance or a multiple doses given within 24 hours. Phychochemical interactions of poisons lead to injury or death of living tissues Toxicology is like science and an art like medicine. It includes observational data gathering & data utilization to predict outcome of exposure in human and animals. The ancient humans categorized some plants as harmful and some as safe. [1] In the recent years, attention has been focused at the traditional (Herbal) way of therapy. It is presumed that Ayurveda Medicines (drugs), which is popular in our country, have lesser side effects as compared to allopathic drugs. Therefore, considerable attention has been directed towards identification of plants with no toxicity that may be used for human consumption.

Terminalia bellerica Roxb. (family: Combretaceae), commonly known as belleric myrobalan and locally known as beheda, is an edible plant found throughout Central Asia. [2] Its fruit has been used in traditional medial system for anemia, asthma, cancer, colic, constipation, diarrhoea, dysuria, headache, hypertension, inflammations, and rheumatism. [3,4] Terminalia bellerica is known to lower the lipid levels in hypercholesterolemic animals.[5] *Moringa pterigosperma* Gaertn. (Moringaceae) Leaves are anti inflammatory, anodyne, anthelmintic, ophthalmic and rich in vitamins A and C. [5] Reports indicate that pharmacological activites of *Moringa pterigosperma* (Gaertn) leaves includes antitumour, radioprotective, antihypertensive, hypoglycemia, diuretic and hypocholestemia activities [6] and diabetes. [7]Cassia Tora L., (Cassia obtusifolia L.), Caesalpiniaceae,is a wild crop and grows in most parts of India According to Ayurveda the leaves and seeds are acrid,laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders. [8]

MATERIAL AND METHODS

Plant material

The *Terminalia bellerica (Roxb.)* fruits were collected from Govali village, Kalyan, Thane district, Maharastra, India. The plant material was taxonomically identified by Blatter Herbarium St Xavier's College, Mumbai. A voucher specimen (No. T-1114 of S.C. Tavakari) has been preserved in a laboratory for further reference. The leaves of *Moringa pterigosperma* (Gaertn) and Cassia Tora

Table 1 : Dosage regimen for acute toxicity study

Group	Sex	Plant	Dose g/kg body weight	No. of animals used	Total volume administered
I	Male	Terminalia bellerica	2.0	4	0.5
II	Male	Terminalia bellerica	5.0	4	0.5
III	Male	Moringa oleifera	2.0	4	0.5
IV	Male	Moringa oleifera	5.0	4	0.5
V	Male	Cassia tora	2.0	4	0.5
VI	Male	Cassia tora	5.0	4	0.5

L., were collected from Birla college campus, Kalyan, Thane district, Maharastra, India. The plant material was taxonomically identified by Blatter Herbarium St Xavier's College, Mumbai. A voucher specimen (No. 4891 of N.A Irani and No. 4455 of N.Y. Das) has been preserved in a laboratory for further reference. The collected plant material was dried under shade and powdered with a mechanical grinder and stored in an air tight container. The dried powder material of was soaked in distilled water and the slurry thus obtained was used further.

Animal maintenance

30 male Swiss albino mice of body weight from 25-30 g were procured from Haffikine Institute, Parel. The animals were housed in polypropylene cages in air conditioned room with controlled temperature and alternating 12 hour periods of light and dark were maintained. The animals were acclimatized to standard laboratory conditions prior to experimentation. The guidelines issued by Institutional Animal Ethics Committee of Ramniranjan Jhunjhunwala College, Ghatkopar, with CPCSEA registration no. 525/02/a/CPCSEA regarding the maintenance and dissection of small animals were strictly followed.

Composition of diet

The aminals were fed on the standard pellet diet (Amurt Feed, Pune), and water was given *ad libitum*. The standard pellet diet comprised 20% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates).

The dosage regimen for acute toxicity study (Table1)

Acute Toxicity Study

Toxicity study of Terminalia bellerica, Moringa oleifera, Cassia tora was carried out by using mice as the experimental model. The study was carried out to assess the acute toxicity of the plant slurry on oral administration. The study was carried out as per the details laid down in OECD guidelines 420 viz, fixed dose procedure (Evident toxicity)

Protocol:-

1. Animal species/ strain	Albino Swiss mice.
2. Sex	Male.
3. Body weight	25–30.
4. Animal procured from	Haffikine Institute, Parel, Mumbai.
5. No. of doses groups	6
6. Animals per group	4
7. Route of administration	Oral via gauage.
8. Vehicle of administration	Distilled water
9. Volume of administration	Not more then 2ml as combined volume of plant sample and vehicle.
10. Dosing details	Refer to dosing chart.
11.Observation period	14 days post dose and 7 days prior to dosing.

More clinical observation such as condition of fur, damage area of skin, subcutaneous swelling or lumps, abdominal detension, eye dullness, eye opacity, pupil diameter, ptosis (drooping of upper eyelid), colour and condition of faeces, wetness or soiling of perineum, condition of teeth, breathing abnormalities, gait should be recorded as indication of toxicity.

Statistical analysis

Experimental results are expressed as means ± SD.

RESULTS AND DISCUSSION

Clinical Observation

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from

the stage of dosing to the end of the study. Any changes or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of whole plant powder as slurry. The clinical observation detailed below is in general for the 3 plants material under investigation.

1.Condition of fur Normal
2.Damage area of skin Normal
3.Subcutaneous swelling or lumps Normal
4.Abdominal detension Normal
5.Eye dullness Normal
6.Eye opacity Normal
7.Pupil diameter Noraml
8.Ptosis (drooping of upper eyelid). Normal
9.Colour and condition of faeces Normal
10.Wetness or soiling of perineum Nil
11.Condition of teeth Normal
12.Breathing abnormalities Normal
13.Gait Nil

1.Body weight changes

Body weight is an important factor to monitor the health of the animal. The loss of body is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10%or more reduction in body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weight for all the 14 days as compared with the 0 day it thus indicating no signs of toxicity (Table 2).

2. Food and water consumption

There was no significant change in food and water consumption(Table 3 and 4).

3. Mortality

Mortality is the main criterion in assessing the acute toxicity (LD50) of a drug. There was no mortality recorded even at the highest dose level i.e.5g/kg body weight of all the groups.

CONCULSION

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the Albino Swiss mice from all the dose groups. There was no mortality recorded even a the highest dose level i.e.5g/kg body weight, which proves that all Terminalia bellirica Roxb., Moringa oleifera Lam.,

Table 2 :. Acute toxicity studies *on Terminalia bellirica* Roxb.(I, II), *Moringa pterigosperma* Gaertn. (III, IV) *Cassia tora Linn.*(V, VI), –Body weight (g)

Days	I	II	III	IV	V	VI
Day 0	30	26	30	26	27	25.8
Day 1	30	26	30	26	27	25.8
Day 2	30	25	29.9	26.1	26.6	25.7
Day 3	29.9	24	29.8	26	26.7	25
Day 4	29	25	30.1	26	26.7	25
Day 5	29	24	30	26	28	25
Day 6	29	26	30	26.5	27	25
Day 7	29.8	26	29	26.4	27	25
Day 8	29.8	26.1	28	26	26.6	25
Day 9	29.9	25.9	28.8	26	27	26
Day 10	29.9	25.5	28.8	27	27	26
Day 11	29.9	26	29	27	27	26
Day 12	29.9	25	30	27	27	26
Day 13	30	26	30	26	27.1	26
Day 14	30	26	31	26	27	26

Note: All values expressed as average weight of animals in each group. The number of animals in each group is four.

Table 1.: Acute toxicity studies on Terminalia bellirica Roxb.(I, II), Moringa oleifera Lam.(III, IV) Cassia tora Linn.(V, VI), –Food intake (g)

Days	I	II	III	IV	V	VI
Day 0	22	17	21	19	16	20
Day 1	16	18	23	18	17	21
Day 2	20	17	21	19	17	21
Day 3	20	16	20	18	19	22
Day 4	18	15	19	19	23	22
Day 5	18	17	18	20	20	22
Day 6	19	18	18	19	18	22
Day 7	19	18	19	18	17	21
Day 8	20	20	18	16	18	15
Day 9	20	20	19	16	16	15
Day 10	20	18	20	16	16	15
Day 11	21	19	20	19	15	15
Day 12	21	17	21	19	15	22
Day 13	22	18	21	18	16	21
Day 14	22	17	21	19	16	21

Note: All values expressed as weight of food consumed by each group,from a known weight of food provided .The number of animals in each group is four.

Table 4.: Acute toxicity studies on Terminalia bellirica Roxb.(I, II), Moringa oleifera Lam.(III, IV) Cassia tora Linn.(V, VI), –Water intake (g)

Days	I	II	III	IV	V	VI
Day 0	21	17	24	20	22	15
Day 1	21	13	23	20	16	17
Day 2	22.3	11	23	20	18	18
Day 3	23	20	24	21	19	16
Day 4	20	17	23	22	18	17
Day 5	19	13	20	23	19	16
Day 6	20	17	20	21	19	14
Day 7	20	20	21	22	21	11
Day 8	18	17	23	23	20	13
Day 9	16	21	20	23	20	14
Day 10	19	19	21	14	15	12
Day 11	18	18	20	19	17	11
Day 12	18	19	20	19	18	13
Day 13	18	17	23	19	18	14
Day 14	17	20	24	19	20	15

Note: All values expressed as ml of water consumed by each group, from a known weight of food provided .The number of animals in each group is four.

Cassia tora Linn. have no toxic effect in Albino Swiss mice. The results have indicated that these plants are safe and can be used for efficacy studies.

REFERENCES

1. Pingale SS. *Acute toxicity study for centella asiatica whole plant powder*. Newsletter, Pharmacologyonline **3**: 80¦84, 2008.
2. Kapoor LD. *Handbook of Ayurvedic Medicinal Plants*. p. 321. CRC Press. Boca Raton, U. S. A. 1990.
3. Duke J A, Bogenschutz-Godwin MJ, Ducelliar J and Duke P A K. *Handbook of Medicinal Herbs*. 2nd ed. pp. 70¦71. CRC Press. Boca Raton, U. S. A. 2002.
4. Usmanghani K, Saeed A and Alam MT. *Indusyunic Medicine*. pp. 420¦421. University Press. Karachi, Pakistan. 1997.
5. Shaila HP, Udupa AL and Udupa SL. *Preventive actions of Terminalia bellerica in experimentally induced atherosclerosis*. *Int. J. Cardiol*. **49**: 101¦106, 1995.
6. Sheth AK. *Moringa oleifera*. The Herbs of Ayurveda. Vol **III** 275¦776, 2005.
7. Hus R, Midcap S, Arbainsyah and DeWitte L.. *Moringa oleifera*; Medicinal and socio-economic uses. International course on Economic botany. National Herbarium Leiden, The Netherlands, 13, 2006.
8. Jaiswal D, Rai PK, Kumar A, Mehta S and Watal G. Effectof *Moringa oleifera* Lam. Leaves extract therapy on hyperglycemic rats. *Journal of Ethanopharmacology*. 123 **3**: 392¦396, 2009.
9. Deore SL, Khadabadi SS, Kamdi KS, Ingle VP, Kawalkar NG, Sawarkar PS, et al. *In vitro* Anthelmintic activity of *Cassia tora*, *International Journal of ChemTech Research*.**2**: 177¦179, 2009

HPTLC Fingerprint Profile Of Some *Cinnamomum* Species

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Abstract

In the present communication, finger print of four medicinally and economically important leaves of *Cinnamomum* species has been developed. Hexane extract of these plants were developed in the mobile phase of toluene : ethyl acetate (8:1, v/v) and scanned under UV at 254 nm and after dipping in vanillin-sulphuric acid reagent followed by heating at 105°. The four species showed differentiating fingerprints on densitomentric scanning at different wavelengths. These finger prints would be helpful in the authentication of these species.

Keywords: *C. malabatrum, C. sulphuratum, C. tamala,* Densitometry, Lauraceae, Petiole.

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INTRODUCTION

About 270 species of *Cinnamomum* Schaeffer (Family: Lauraceae) are distributed in Asia, America and Australia out of which 20 specie occur in South India.[1–2] Several Cinnamomum species are medicinally and economically important. The genus plays an important role in the search for new source of aroma chemicals and it is a prime area where perfume industries are interested.[3–4] Investigation of *Cinnamomum* species lead to the discovery of aroma chemicals having unique flavor due to aroma chemicals like eugenol, linalool, safrole and benzyl benzoate.[5–7] The aroma principles eugenol and linalool possess different biological properties.[9–15] Four economically important species viz., *C. malabatrum, C. sulphuratum, C. tamala* and *C. zeylanicum* were taken up for the HPTLC finger print studies using eugenol and linalool as appropriate chemical markers.

MATERIALS

Leaves of *Cinnamomum* viz. *C. malabatrum, C. sulphuratum, C. tamala* and *C. zeylanicum* growing in the evergreen forests of Coorg district of Karnataka were collected in the month of January and were authenticated with the help of Regional floras.[17] The leaves were shade dried and the petioles were separated from the lamina portion and used for the study. Analytical reagents *viz., n*-hexane, toluene, ethyl acetate were purchased from SRL chemicals, Mumbai, India. TLC plates were obtained from Merck, Bombay, India. Eugenol and linalool were obtained from M/s. Sigma-Aldrich chemicals, Bangalore, India.

METHODS

1 g of finely crushed petiole of the selected plants were separately extracted with *n*-hexane using Soxhlet apparatus. The extracts were freeze dried. The residues were dissolved in 10 ml of *n*-hexane. 10 mg of each of eugenol and linalool were dissolved in 10 ml of *n*-hexane.
TLC aluminium plates precoated with silica gel 60 F_{254} (Merck) of 0.2 mm thickness was used as stationary phase. 10 µl of *C. malabatrum, C. sulphuratum, C. tamala*, 20 µl of *C. zeylanicum* and 4 µl of eugenol and linalool were applied as 6 mm bands at 6 mm distance on the TLC plates using CAMAG Linomat IV sample applicator. The speed of the application of extracts was maintained at 5 sec/µl. Then the plate was developed using the mobile phase of Toluene : Ethyl acetate (8:1, v/v) in a CAMAG twin trough chamber up to a height of 8 cm. The developed plate was air dried and scanned at a wavelength of 254 nm using deuterium lamp in the CAMAG scanner 030618 equipped with CATS V 4.06 software. The chromatograms were recorded. Then the plate was dipped in vanillin-sulphuric

acid reagent. The plate was heated in an air circulated oven at 105°C till the development of colour of the spots. Then the plate was scanned immediately in the visible region at a wavelength of 620 nm using tungsten lamp. UV spectrum of marker compounds and their corresponding spots in the extracts were also recorded.

RESULTS

From the HPTLC finger prints, the peak of eugenol was found at R_f 0.69 and linalool was found at R_f 0.57. The chromatograms obtained were shown in Fig. 1–11. The R_f of peaks under UV 254 nm were listed in Table 1. The R_f of peaks under visible light at 620 nm were listed in Table 2. Peaks with R_f value less than 0.05 and higher than 0.96 were not included in the table as the former represents the point of application and latter represents the movement of the constituents to the solvent front.

DISCUSSION

In the chromatograms derived under UV 254 nm (Fig. 1-5), the peaks at R_f 0.73 in all four plant extracts corresponds to eugenol and linalool was not visible in UV light. The peak area of eugenol in *C. zeylanicum* was comparatively higher than other species. Peaks at R_f 0.25, 0.33, 0.63, 0.73, 0.87 & 0.95 were common to *C. sulphuratum* and *C. tamala*; but peaks at R_f 0.25 was minor in *C. sulphuratum* whereas major in *C. tamala*. Peak at R_f 0.43 of *C. sulphuratum* was specific to this specie and it was not seen in other species. Similarly, a major peak at R_f 0.37 of *C. tamala* was found to be specific to this plant.

Table 1. RF VALUES OF PEAKS OBSERVED UNDER UV 254 NM

Sl.No.	Cm	Cs	Ct	Cz	Marker
1	-	0.06 (Minor)	-	-	
2	-	0.10(Minor)	0.09(Minor)	-	
3	-	-	0.14(Minor)	-	
4	-	0.25(Minor)	0.25(Major)	-	
5	-	0.33(Minor)	0.33(Minor)	-	
6	0.39(Minor)	-	0.37(Major)	-	
7	-	0.43(Major)	0.46(Minor)	-	
8	0.59(Minor)	-	-	-	
9	-	0.63(Major)	0.63(Major)	-	
10	0.73(Minor)	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Eugenol)
11	-	0.87(Major)	0.87(Major)	0.86(Minor)	
12	0.95(Major)	0.95(Major)	0.94(Major)	0.94(Minor)	

Table 2. RF VALUES OF PEAKS OBSERVED UNDER UV 620 NM

Sl. No	Cm	Cs	Ct	Cz	Marker
1	0.05	-	-	0.07	
2	0.13	0.13(Major)	0.13(Minor)	0.13	
3	-	0.18(Major)	0.18(Minor)	-	
4	0.25	0.25(Major)	0.25(Major)	0.27	
5	-	0.29(Minor)	0.32(Minor)	0.31	
6	0.31(Major)	-	0.39(Major)	0.40(Major)	
7	0.40(Major)	0.41(Major)	-	-	
8	-	0.51(Major)	-	-	
9	0.57(Major)	0.57(Major)	0.57(Major)	0.57(Major)	0.57(Linalool)
10	-	0.64(Major)	0.66(Minor)	-	
11	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Major)	0.73(Eugenol)
12	-	0.78(Major)	0.78(Major)	0.78(Major)	
13	-	-	-	0.93(Major)	

Cm- Cinnamomum malabatrum; Cs- Cinnamomum sulphuratum; Ct-Cinnamomum tamala; Cz- Cinnamomum zeylanicum

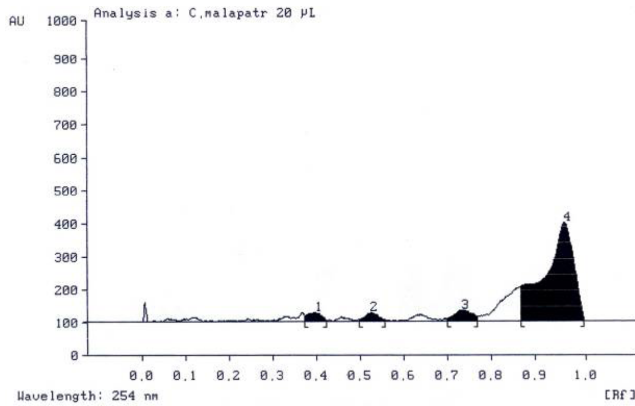

FIGURE 1. *FINGER PRINT OF C. MALABATRUM AT • 254 NM*

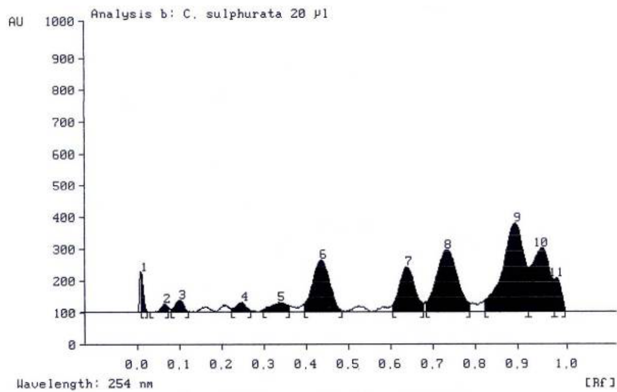

FIGURE 2. *FINGER PRINT OF C. SULPHURATUM AT • 254 NM*

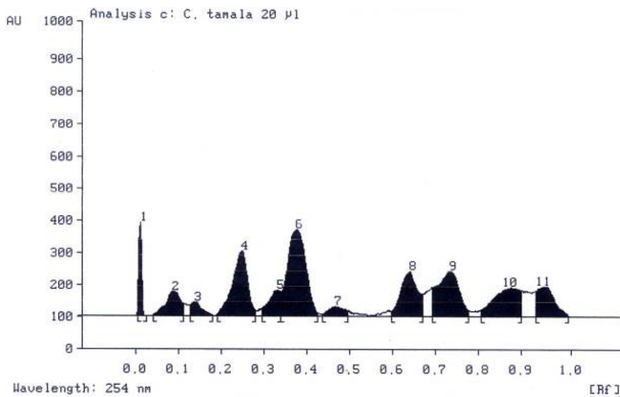

FIGURE 3. *FINGER PRINT OF OF C. TAMALA AT • 254 NM*

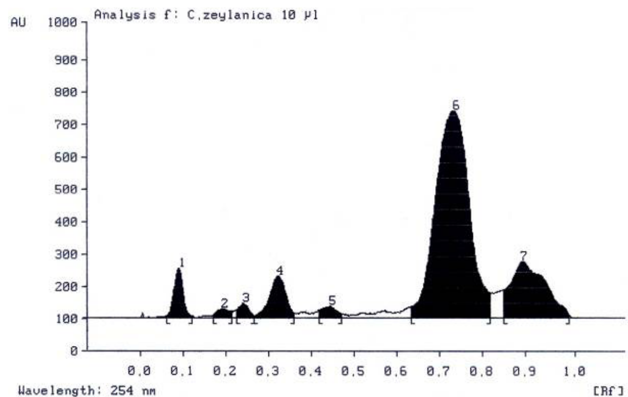

FIGURE 4. *FINGER PRINT OF OF C. ZEYLANICUM AT • 254 NM*

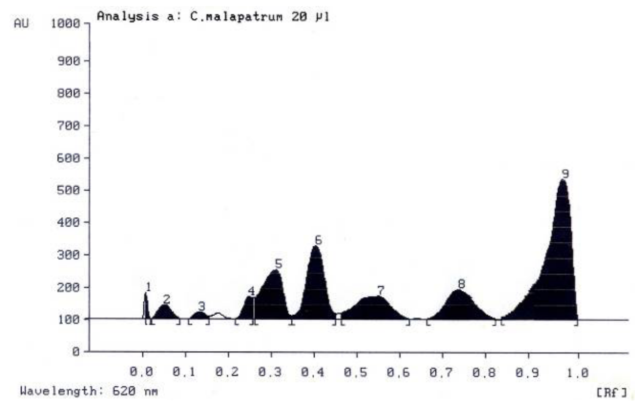

FIGURE 5. *FINGER PRINT OF OF C. MALABATRUM AT • 620 NM*

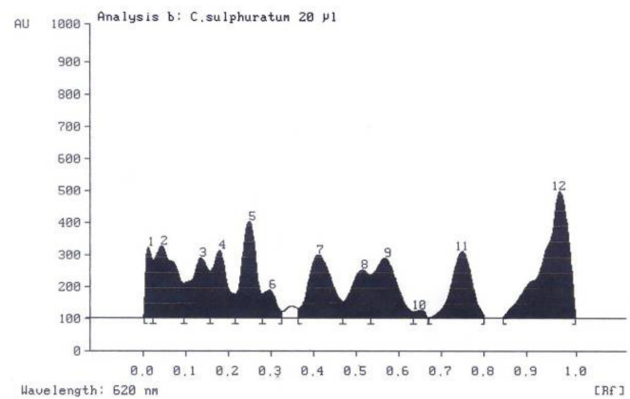

FIGURE 6. *FINGER PRINT OF C. SULPHURATUM AT • 620 NM*

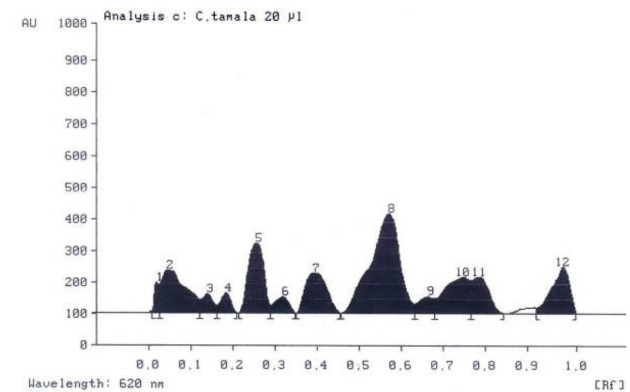

FIGURE 7. *FINGER PRINT OF C. TAMALA AT • 620 NM*

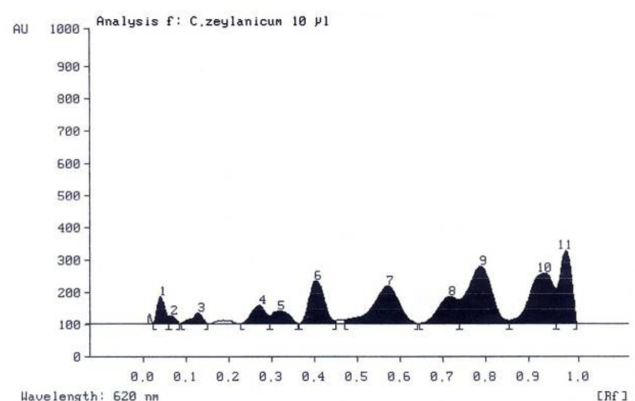

FIGURE 8. *FINGER PRINT OF C. ZEYLANICUM AT • 620 NM*

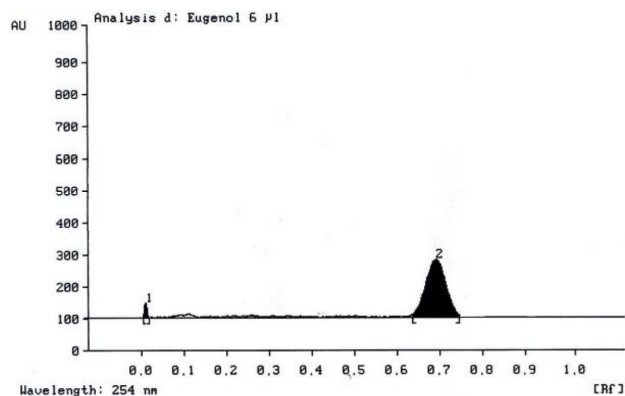

FIGURE 9. *FINGER PRINT OF C. EUGENOL AT • 254 NM*

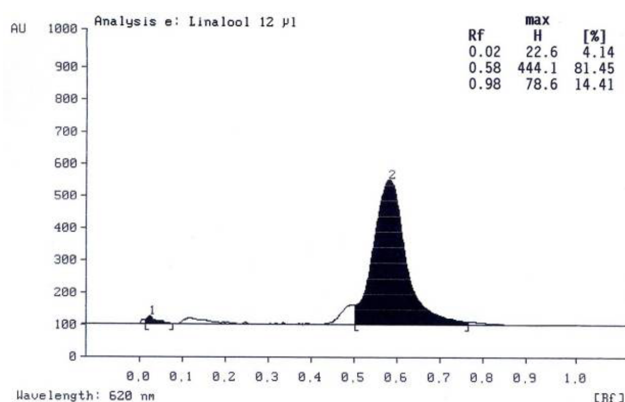

FIGURE 10. *FINGER PRINT OF LINALOOL AT • 620*

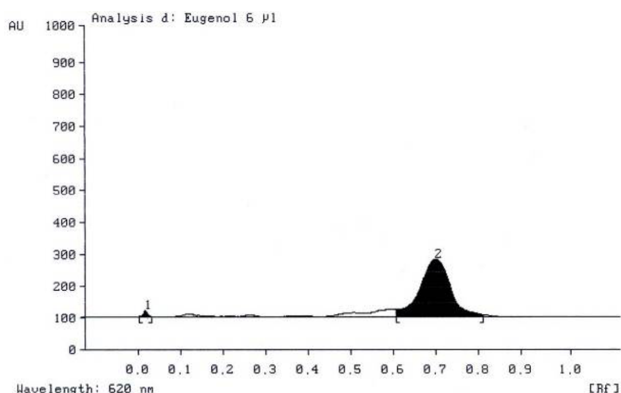

FIGURE 11. *FINGER PRINT OF EUGENOL AT • 620 NM*

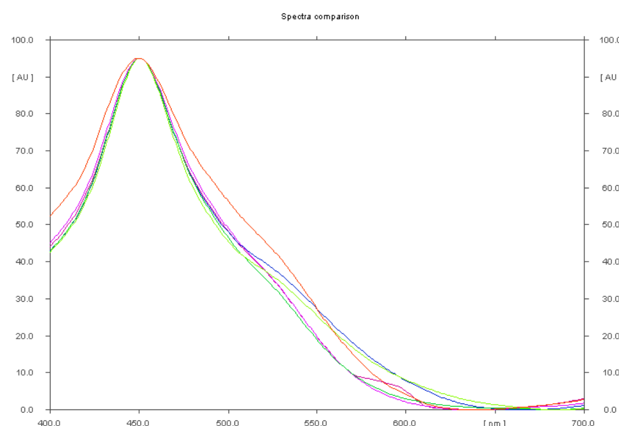

FIGURE 12. *SUPERIMPOSABLE UV SPECTRA OF EUGENOL*

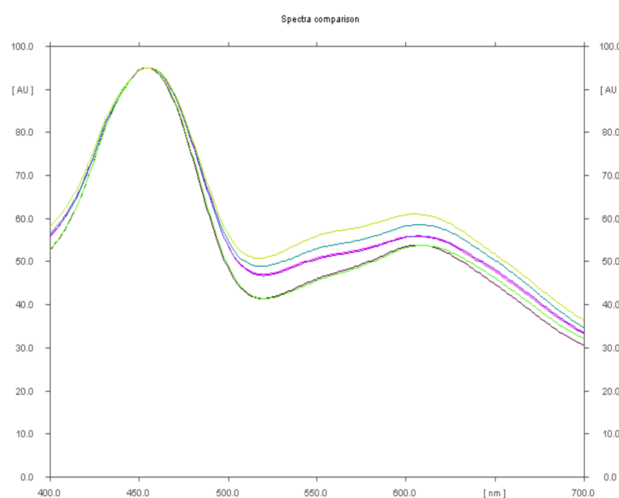

FIGURE 13. *SUPERIMPOSABLE UV SPECTRA OF LINALOOL*

In the chromatograms derived under visible light at 620 nm (Fig.6-11), peaks at R_f 0.57 and 0.73 corresponds to linalool and eugenol respectively and their UV super imposable spectra were shown in Fig.12 & 13. *C. malabatrum* showed 7 spots and other species showed 9 peaks each. Peak at R_f 0.13 was common in all species; but major in *C. sulphuratum* and minor in others. Similarly, peak at R_f 0.25 was common in all species but major in *C. sulphuraturm* and *C. tamala*. Peak at R_f 0.31 was major in *C. malabatrum* whereas it is minor in other species. Minor peak at R_f 0.64 of *C. sulphuratum* and 0.66 of *C. tamala* were comparable. Peak at R_f 0.93 of *C. zeylanicum* is specific to it.

CONCLUSION

C. sulphuratum and *C. tamala* were comparable to each other. The solvent system used in the study was able to differentiate the four species.

ACKNOWLEDGEMENT

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REFERENCES

1. Kostermans AJGH. *The South Indian species of Cinnamomum Schaeffer (Lauraceae)*. Bull Bot. Surv. India. 1986; **25**: 90-133.

2. Cooke TCIE. *Flora of the presidency of Bombay*. London: Taylor & Francis. Vol.**1**; 1903; 282–283.
3. Senanayake VM, Wijesckera ROB. *The volatiles of the Cinnamomum species, Proceedings of the 11th International Congress of Essential Oils*. Frag and Flav. 1990; **4**: 103–120.
4. Nath SC, Baruah A, Hazarika AK. Essential oils of the leaves of *Cinnamomum* Schaefer members. *Indian Perfumer*. 1999; **43**: 473–478.
5. Hussain RA, Kim J, Hu TW, Soejarto DD, Kinghorn AD. Isolation of highly sweet constituent from *Cinnamomum osmophloem* leaves. *Planta Med*. 1986; **5**: 403–404.
6. Nath, S.C.; Baruah, K.S. Eugenol as the major component of the leaf oils of *Cinnamomum impressinervium* Meis. *J Essent. Oil Res*. 1994; **6**: 211–212.
7. Zhou R. *A new species of safrole containing plant Cinnamomum petrophilum N. Chao. In Proceedings of 9th International Congress of Essential Oils, Book 1*, Essential Oils Association of Singapore: Singapore; 1983; 102–103.
8. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine* 2002; **9**: 721–726.
9. Maria do Socorro S, Rosa Mendonca-Filho RR, Bizzo HR, Igor de Almeida R, Soares RMA, Souto-Padron T, Alviano CS, Lopes AHCS. Antileishmanial Activity of a Linalool-Rich Essential Oil from *Croton cajucar*. *Antimicrobial Agents and Chemotherapy*. 2003; **47**: 1895–1901.
10. Peana AT, Marzocco S, Popolo A, Pinto A. (-)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sci*. 2006; **78**: 719–23.
11. Perrucci S, Cioni PL, Cascella A, Macchioni F. Therapeutic efficacy of linalool for the topical treatment of parasitic otitis caused by *Psoroptes cuniculi* in the rabbit and in the goat. *Medical and Vet. Entomol*. 2008; **11**: 300–302.
12. Benencia F, Courrèges MC. *In vitro* and *in vivo* activity of eugenol on human herpesvirus. *Phytother. Res*. 2000; **14**: 495–500.
13. Pisano M, Pagnan G, Loi M, Mura ME, Tilocca MG, Palmieri G, Fabbri D, Dettori MA, Delogu G, Ponzoni M, Rozzo C. Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Molecular Cancer*. 2007; **6**: 8.
14. Ogata M, Hoshi M, Urano S, Endo T. Antioxidant Activity of Eugenol and Related Monomeric and Dimeric Compounds. *Chem. Pharm. Bull*. 2000; **48**: 1467–1469.
15. Guenette SA, Beaudry F, Marier JF, Vachon P. Pharmacokinetics and anesthetic activity of eugenol in male Sprague–Dawley rats. *J. Vet. Pharmacol. & Therapeut*. 2006; **29**: 265–270.

Collection and storage of medicinal plants in The Canon of Medicine

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Abstract

The *Canon of Medicine* was the reference of medical sciences for many years in western and eastern countries. The second book of this medical encyclopedia is on pharmacology which has a chapter on collection and storage of plant materials. In this work a comparison was done on the *Canon of Medicine* and modern rules in this area. It seems that Ibn Sina had developed ideas on the condition of plant collection and storage which guaranteed the quality of primary materials. Overall, his rules and modern ones are alike in the most parts.

Keywords: Ibn Sina, Avicenna, Canon of Medicine, Plant collection and storage.

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INTRODUCTION

The *Canon of Medicine* is one of almost 450 treatises written by the Persian scientist and physician Ibn Sina (Avicenna).[1] It remained a medical sciences authority up until the 18th century and early 19th century.[2] The book was translated into Latin, Hebrew, French, English and Chinese.[1] Ibn Sina divided his *Canon of Medicine* into five books. The first book concerns basic medical and physiological principles as well as anatomy, regimen and general therapeutic procedures. The second book is on the pharmacology of medical substances, arranged alphabetically, following an essay on their general properties. The third book concerns the diagnosis and treatment of diseases specific to one part of the body, while the fourth covers conditions not specific to one body part, such as poisonous bites and obesity. The final, fifth, book is a formulary of compound remedies. The 2nd book lists 800 tested drugs, including plant animal and mineral substances, with comments on their application and effectiveness. Ibn Sina begins the second book with a discussion on the nature and quality of drugs and the way that mixing them influences their effectiveness.[3] The 6th chapter of Book 2 is "The collection and storage of drugs".

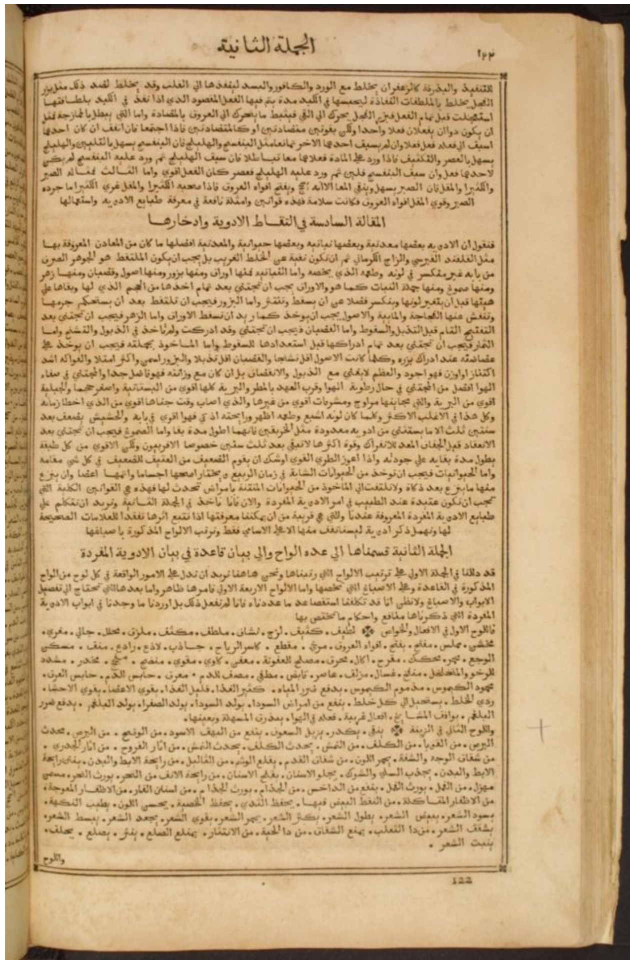
This article presents text from the *Canon of Medicine* which discusses about collection and storage of medicinal plants and makes a comparison between these texts with current pharmacognosy literature.

MATERIAL AND METHOD

For investigation of Avicenna's Canon, the electronic manuscript which is available in American University of Beirut library[4] (Figure 1)and also the Persian translation of Canon[5] were used. The section of *collection and storage of drugs* from each book was investigated, compared and also translated in to English. Furthermore, two textbooks of pharmacognosy, *Trease and Evans Pharmacognosy*[6] and *Drugs of Natural Origin,*[7] were considered as the main references of modern pharmacognosy. Finally, Avicenna's theories were compared with the modern ones.

RESULTS AND DISCUSSION

According to the *Canon of Medicine* the parts of the herbs that can be used as medicine include leaves, roots, branches, seeds, fruits, flowers, and gums. Most of these parts are the same as those are used today (Table 1).



الجملة الثانية

المقالة السادسة في التقاط الادوية وادخارها

Figure 1 The plant collection and storage part of the second book of the *Canon of Medicine* in Arabic on bladder. Adapted from the web site of the Saab Medical Library of the American University of Beirut.

Ibn Sina considered some special conditions for plant collection. He said: "It is essential that the herbs to be procured should be fresh and seeds should have formed within them, their roots should not be deformed or old, the seeds should be mature and not shriveled. The best fruit are those which have attained full size and weight. Nuts which are shriveled or broken are of no advantage; better among them are those which have acquired their full weight".[4]

Table 1 shows that the leaves should be collected before discoloration and in the time of complete growth which seems to be the flowering stage. About seeds, fruits, branches and flowers traditional and modern opinions confirm each other (Table 1). For gums they should be collected when coagulated but they are not so much hard that they begin to be frittered away.

Ibn Sina, of course, considered some criteria for perfect growth like for seeds that should be totally hard and without moisture; fruits should be plucked before they fell down; branches when they have reached perfection and have not started drying or withering. The interesting point is about roots. Ibn Sina knew that the time of compound accumulation in roots is in autumn and at the end of vegetation period or in his term "when the Plants are on the threshold of shedding their leaves".[4]

He also believed that the plants which are procured when the weather is clear are better than those which are collected when the weather is humid or the rainy season is near. This idea is completely in conformity with what is mentioned in new texts where it said that leaves, flowers and fruits should not be collected when covered with rain.[6]

Table 1. A comparison between time of collection of plant parts between *Canon of Medicine* and Modern literature.

Part of the herb	Time of collection	
	Ibn Sina's theory	**Modern theory**
Flowers	When they have reached full bloom but have not dried up or fallen down.	Just before they are fully expanded [6], When fully developed [7].
Fruits	When fully matured but before they fall down.	When fully ripped[7].
Leaves	When they have attained their full size. The withered, discolored, shattered and also fallen ones should not be taken.	As the flowers are beginning to open[6], at the flowering stage [7].
Roots	When the plants on the threshold of shedding their leaves.	As the aerial parts die down[6], at the end of vegetation period, i.e. usually in the autumn [7].
Seeds	When their mass condensed and their rawness and moisture have been disappeared.	When fully ripped[7].

He also mentioned that the plants growing on hills or mountains are stronger than those growing in plains. Drugs collected from forests and places which are exposed to the sun-ray are better than those from other (shady) places. In Ibn Sina's opinion the herbs growing wild are stronger than those which are cultivated and those collected at appropriate times are better than those collected at inappropriate times. He mentioned that all those herbs which have a deep color, definite taste and distinct smell are stronger.

According to the *Canon of Medicine*, the effectiveness of dry plant materials would decrease after three years, it is similar to what new references offer about the shelf life of medicinal plants.[8]

Nowadays, plant collection and storage is an important part of herbal medicine industry.[9] It seems that Ibn Sina was completely acquainted with the fact that the existing material in plants are different in various seasons, times and places and also long time preservation of the herbs would result in reduction of active constituents. So he posed some rules about collection and storage of medicinal plants. By considering these rules, one can realize the Ibn Sina's precision and meticulosity about preparation of appropriate primary materials which cause in quality improvement of final products and repeatability of drug effects.

REFERENCES

1. Koh G. The Canon of Medicine (Al-Qanun fi'l-tibb) By Ibn Sina (Avicenna) 11th century. *BMJ* 2009; **339**: b5358.
2. Wikipedia. *The Canon of Medicine" (work by Avicenna)*. Wikipedia, from ext-link-type="uri" xmlns:xlink="http://www.w3.org/1999/xlink" xlink:href="http://en.wikipedia.org/wiki/The_Canon_of_Medicine">http://en.wikipedia.org/wiki/The_Canon_of_Medicine, 2010.
3. Nasser M, Tibi A, Savage-Smith E. "Ibn Sina's Canon of medicine: 11th century rules for assessing the effects of drugs. *Journal of the Royal Society of Medicine* **2009**;**102**: 78–80.
4. Ibn Sina AA. *Kitab al Qanoun fi Al Toubb, al-Kitab al-Sani [The Book of Canon of Medicine, Book 2] Arabic*. Romae, Typographia Medicea, 1593.
5. Avicenna. *Al-Qanun fi al-tibb*. Tehran, Soroush, 2004.
6. Evans WC. *Trease and Evans Pharmacognosy*. Edinburg, WB Saunders. 2002.
7. Samuelsson G. Drugs of Natural Origin, A Text Book of Pharmacognosy Stockholm, Swedish Pharmaceutical Press, 1999.
8. Brown G. *Herbs and spices*. [cited 19 January, 2010]. Available from: http://www.wvu.edu/~exten/infores/pubs/fypubs/wl107.pdf, (1995).
9. Heinrich M, Barnes J, Gibbons S, Williamson EM. *Fundamentals of Pharmacognosy and Phytotherapy*. Edinburg, Chirchil Livingstone, 2004.

Antioxidant Activity of Fruit Pulp Powder of *Cassia fistula*

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Abstract

Fruit pulp powder of *Cassia fistula* was investigated for its antioxidant activity both *in vitro* and *in vivo*. Preliminary phytochemical analysis showed high phenolic and flavone content (22 mg/kg and 4 mg/kg respectively) in pulp. A concentration dependent, increase in FRAP value obtained; suggest high antioxidant property of the extract. *In vivo* study in young adult mice fed fruit pulp powder extract (100 mg/ kg/BW single dose daily for 30 days) one hour prior to Combination of stresses (immobilization followed by swimming type) of 2 hrs duration daily up to 30 days, showed significant increase in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) levels in brain, gastronomies muscle, heart, kidney, lung and stomach of these mice as compared to mice given only stress. Levels of malondialdehyde (MDA) were significantly lowered after the drug treatment in all the tissues in stress group mice as compared to control. High antioxidant activity of *Cassia fistula* may be contributed to its high phenolic and flavonoid content.

Keywords: Antioxidant, *C. fistula*, free radicals, mice.
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INTRODUCTION

Cassia fistula Linn. (Leguminosae) is commonly known as Amaltas, Indian Laburnum or pudding pipe tree and Aragvadha. The plant is found in India, Pakistan, Bangladesh, Tropical Africa, South America and the West Indies. It is native of tropical climatic area and is present all over Asia. It is also present in the westem tropical region of America. It grows freely all over India. It is a medium sized deciduous tree that reaches the height of aboot 25 to 30 feet. The bark is of reddish brown in color. Leaves are alternate, pinnate, 1 to 1.5 feet long and possess pairs of four to eight ovate leaftets aboot 7 to 15 centimeter long and neariy 2, 5 to 3 centimeter broad, This entire petiole is about 3 to 7 millimeter long. It bears yellow colored flowers. That droops down depending upon the length of the raCemes. The fruit beared by the amallas tree is pendulous, cylindrical, brown and septate having a length of 25 to 45 centimeters and possess a diameter of 1 to 3 centimeters. It has within it aboot 30 to 100 seeds, Seeds are lenticular with red-brownish texture. Tree sheds its leaves in March and April. Fruits arise by rainy season[1]. Preparation of bark, leaves and flowers are commonly used as household medicine for various aliments.

Leaves of *Cassia fistula* shown to possess *antitussive* and wound healing properties[2,3]. Antitumor property has also been shown by the seed extract[4]. Bark of this plant is reported to possess anti-malarial [5]. and Anti-inflammatory and antioxidant activities[6]. Similarly antioxidant activity of its flowers has been reported in alloxan induced diabetic rats[7]. Its antioxidant activity is suggested because of the presence of flavanoids[8,9]. Rhein glycosides, sinoside A and B in pods are also isolated. A new bioactive flavone glycoside 5,3′,4′-tri-hydroxy-6-methoxy-7-O-alpha-L-rhamnopyranosyl-(1 --> 2)-O-beta-D-galactopyranoside with antimicrobial activity was reported by Yadav and Verma [9]. Four new compounds,

5-(2-hydroxyphenoxymethyl)furfural, (2′S)-7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl) chromone, benzyl 2-hydroxy-3,6-dimethoxybenzoate, and benzyl 2beta-O-D-glucopyranosyl-3,6-dimethoxybenzoate, together with four known compounds, 5-hydroxymethylfurfural, (2′S)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone, and two oxyanthraquinones, chrysophanol and chrysophanein, were also isolated from the seeds of *Cassia fistula* by Kuo et al.,[10]. *Cassia fistula* is rich in tannin, anthraquinones, rehein I, emodin II, kaempferol and roanthocyanidin[11,12].

Although various parts i.e., leaves, bark, seed etc., have been studied for their antioxidant property and thus of high medicinal value, but review of literature reveal that pod pulp of the *C. fistula* has not been studied in spite of its common use in Indian household medicines for various ailments. Oxidative stress is an important contributor to the patho-physiology of variety of diseases, including cardiovascular dysfunctions, cancer, diabetes, atherosclerosis, inflammatory diseases and neurodegenerative diseases etc.,[13,14]. Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect cells and their constituents against reactive oxygen species (ROS) induced damage[15]. As the innate defense is not enough against severe or continuous oxidative stress, exogenous antioxidants are required. Although many synthetic antioxidants are in use but many of them have side effects, hence compounds from natural resources need to be identified having potency to prevent ROS induced damage.

In present paper, we have reported the preliminary evidence of antioxidant activity of the pulp powder extract of *C. fistula*. FRAP assay (Ferric reducing antioxidant power assay) was carried in *vitro*. Antioxidant profile i.e., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), and Malondialdehyde (MDA) were studied in brain, liver, kidney, muscle, heart, intestine and lung of animals fed pulp powder extract.

MATERIAL AND METHOD

Chemicals:

Sodium acetate trihydrate, acetic acid, EDTA, Pot. Dichromate, $FeCl_3$ purchased from Hi media (Mumbai, India). Sodium, Sodium Azide, Sodium hydrogen phosphate, Hydrogen peroxide, Glutathione, Di nitrobenzoic acid, Riboflavin, Sodium cyanide, Nitro blue tetrazolium were purchased from SRL, New Delhi, India. Ascorbic acid, 2,4,6-tripyridyl-s-trazine (TPPZ), from Sigma, USA.

Plant collection and identification and extraction

C. fistula pods were collected from trees growing by the roadside in Udaipur, India and were identified by Prof. K.G. Ramawat of the Department of Botany, Mohan Lal Sukhadia University (Udaipur, India). A voucher specimen of the pod was deposited at the herbarium of the department. Pulp was extracted manually from the dried pods by scraping and shade dried at room temperature and ground in an electrical grinder and passed through sieve number 240 to obtain a fine powder of mesh size 60. Soaking 20 gm pulp powder in 100 ml absolute ethanol for 24 h and filtering it through Whatman paper ethanol extract was prepared. Vaccuoevaporation yielded 18.35 % w/w of dried extract. Dried extract was stored in the refrigerator till further use.

Phytochemical screening

Total phenolic content and presence of flavonoids in alcohol extract was estimated by methods suggested by Tanner and Brunner [16] and Zhishen et al., [17] respectively.

Ferric reducing antioxidant power (FRAP) assay

Antioxidant activity of *C. fistula* fruit pulp extract was determined by FRAP Assay method of Banzie and Strain[18]. FRAP assay uses antioxidants as reluctant in a redox-linked colorimetric method, employing an easily reduced oxidant system. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (Which has an intense blue colour) can be monitored, by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

In vivo studies

Departmental Ethical committee as per CPCSEA (Govt. of India) norms (Approval No 973/ac/06/CPCSEA) cleared experiments. Young adult (one month old) Swiss albino inbred mouse strain (n = 40) was used for the study. Animals were randomized and divided into Control (C) and three Experimental groups (E-I, E-II and E-III), each comprising of ten animals and maintained in standard laboratory conditions. Control animals were fed normal diet. Animals of E-I group were subjected to 2 hrs combination of stress (immobilization followed by swimming), daily up to 30 days. This practice was necessary to facilitate the absorption of drug through gastro intestinal tract. E-II animals were fed pulp powder

(100 mg/kg/BW) daily and after one hour were subjected to similar stress. E-III animals were fed pulp powder but were not subjected to stress.

Preparation of tissue and assay of SOD/CAT/GPx/GSH/MDA:

Animals were decapitated and brain, gastronomies muscle, heart, intestine, kidney, lung, liver and stomach were dissected out and placed on chilled glass plate. Each organ was minced thoroughly with the surgical blade. The minced tissue was homogenized thoroughly for 15 min and centrifuged at 10,000 rpm for 15 min. The supernatant was used for assay of SOD [19], CAT [20]; GPx [21]; GSH [22]and MDA[23].

Statistical Analysis:

One way Analysis of Variance (ANOVA) was used to compare the means of all 4 groups. Tukey's Multiple Comparison Test (Post hoc) test was applied to identify pairs of groups, which differed significantly when the omnibus ANOVA was significant. The Tukey's Multiple Comparison Test (Post hoc) test was applied because all the groups were having equal number of replicates. All the statistical test were performed by using the program GraphPad Prism 3 to assess the effect of experimental condition.

RESULTS

Preliminary phytochemical screening of the extract of *C. fistula* pulp powder revealed the presence of various bioactive components of which phenolic (22mg/kg) and flavonoid (4mg/kg) were most prominent.

In present study, a concentration dependent increase in FRAP values for the pulp powder extract were observed (Table.1). The free radical scavenging activity of the extracts was evaluated based on the ability to reduce the ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. Reduced ferrous form of ferric tripyridyl triazine shows a strong absorption band at 593 nm in visible spectrum (deep blue colour). As the electron became paired of in the presence of free radical scavenging, the absorption increases and the resulting intensity of color stoichiometrically coincides with respect to the number of electrons taken up. The increased absorption is representative of the capacity of the test drugs to scavenge free radicals independently.

Table-1: Antioxidant Activity of the Fruit Pulp Extract by FRAP Assay.

S. No.	Sample Concentration (Fruit Pulp)	FRAP (μ mol/kg)
1	0.1%	228
2	1.0%	1028
3	10.00%	10280

Superoxide Dismutase (SOD):

Transfer of a single electron to O_2 generates the potentially damaging superoxide anion free radical (O_2–), the destructive effects of which are amplified by its giving rise to free radical chain reactions. The ease with which superoxide can be formed from oxygen in tissues and the occurrence of Superoxide dismutase, the enzyme responsible for its removal in all aerobic organisms (although not in obligate anaerobes) indicate that the potential toxicity of oxygen is due to its conversion to superoxide.

Application of one way ANOVA depicts that levels of Superoxide Dismutase altered significantly after the stress and dose treatment (F=43.5, P<0.0001) in brain. Tukey's Multiple Comparison Test (Post hoc) reveled that there is a significant difference between the groups for Brain (Control and stress MD= 58.9, P<0.001; Control and Stress+Dose MD= 20.0, P<0.05; Stress and Stress+Dose MD= -38.9, P<0.001; Stress and Dose MD= -62.8, P<0.001; Stress+Dose and Dose MD= -23.9, P<0.01) except when control was compared to dose (MD= -3.9, P>0.05). Similarly when SOD content was analyzed in stomach One way ANOVA depicted significant difference (F=59.2, P<0.0001). Post hoc analysis between the experimental and control groups exhibited that superoxide dismutase altered significantly (Control and stress MD= 56.7, P<0.001; Control and Stress+Dose MD= 32.2, P<0.05; Stress and Stress+Dose MD= -24.4, P<0.001; Stress and Dose MD= -44.4, P<0.001; Stress+Dose and Dose MD= -20.0, P<0.01). Expected non significant difference was found between control and dose (MD= 12.2, P>0.05). SOD content in Intestine was significantly different (F=13.9, P<0.0001). Comparison between groups illustrates that non significant mean difference between Control and Dose (MD= 15.0, P>0.05) Stress+Dose and Dose (MD=-11.1, P>0.05). Rest of all other groups exhibited significant mean difference (Control and Stress MD=55.0, P<0.001; Control and Stress+Dose MD=26.2, P<0.05; Stress and Stress+Dose MD= -28.9, P<0.05; Stress and Dose MD= -40.0, P<0.001). Superoxide Dismutase content difference in lungs was significant (F=46.8, P<0.0001). Comparison of groups when analyzed with post hoc test was found to

be significantly different in all the groups (P<0.001). SOD concentration in Liver was significantly different (F=514, P<0.001). Tukey's Multiple Comparison Test reveled that mean difference between the experimental and control groups was significant except Control Vs Dose (MD= 3.86, P>0.05). Comparison between all other groups was significant (P<0.001). Analysis of Kidney reveled significant difference (F=236, P<0.001). Post hoc analysis exhibited significant mean difference between all groups. Control and Stress, Control and Stress+Dose, Control and Dose, Stress and Stress+Dose were highly significant (P<0.001). Stress and Dose, Stress+Dose and Dose were significant (P<0.01). SOD concentration in Muscle was significantly different (F=333, P<0.001). Tukey's Multiple Comparison Test reveled that mean difference between the experimental and control groups were significant (P<0.001). Superoxide Dismutase content difference in Heart was significant (F=100.0, P<0.0001). Comparison of groups when analyzed with post hoc test was found to be significantly different in control and experimental groups. Control Vs Stress (MD= 51.7, P<0.001); Control Vs Stress+Doe (MD=10.6, P<0.05), Stress Vs Stress+Dose (MD= -41.1, P<0.001); Stress Vs Dose (MD= -47.8, P<0.001). Comparison of Control and Dose (MD= 3.89, P>0.05) Stress+Dose Vs Dose (MD= -6.66, P>0.05) was non significant.

Catalase (CAT):

Hydroperoxidases use hydrogen Peroxide or an organic peroxide As substrate. Two type of enzymes found both in animals and plants fall into this category: peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides. Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and plays role in causation of disease.

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor. Under most conditions in vivo, the peroxidase activity of catalase seems to be favored. Catalase is found in blood, bone marrow, mucous membranes, kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases.

Effect of stress and dose treatment altered catalase levels significantly. After stress treatment catalase levels exhibited decrease in concentration and when stressed animals were treated with dose levels of enzyme were commensurate to control animals. This trend was pervasive in all tissues and was significant although post hoc analysis depicted variations in significance level.

Application of one way ANOVA depicts that levels of Catalase altered significantly after the stress and dose treatment (F=204, P<0.0001). Tukey's Multiple Comparison Test (Post hoc) reveled that there is a significant difference between the groups for Brain (Control and stress MD= 29, P<0.001; Control and Stress+Dose MD= 5.0, P<0.05; Control and Dose MD= -9.25, P<0.001; Stress and Stress+Dose MD -24.0, P<0.001; Stress and Dose MD -38.3, P<0.001; Stress+Dose and Dose MD= -14.3, P<0.001). When CAT content was analysed in stomach One way ANOVA resulted into significant difference (F=269, P<0.0001). Post hoc analysis between the experimental and control groups exhibited that change in catalase concentration was significant (Control and stress MD= 40.5, P<0.001; Control and Stress+Dose MD= 17.3, P<0.001; Stress and Stress+Dose MD= -23.3, P<0.001; Stress and Dose MD= -39.5, P<0.001; Stress+Dose and Dose MD= -16.3, P<0.001). Non significant difference was found between control and dose (MD= 1.0, P>0.05). Analysis of Catalalase content inferred that there is significant difference (F=248, P<0.0001). Post hoc test between control and experimental groups upshot into significant difference between groups, excluding Control Vs Stress+Dose (MD=2.25, P>0.05). Catalase activity was significantly decreased in lungs after stress treatment and up concentration trend was found after drug treatment and all this was found to be significant after one way ANOVA (F=145, P<0.0001). Post hoc analysis represented significant mean difference among the groups (Control Vs Stress MD=30.5,P<0.001; Control Vs Stress+Dose MD= 13.8, P<0.001; Stress Vs Stress+Dose MS= -16.8, P<0.001; Stress Vs Dose MD= -33.8,P<0.001; Stress+Dose Vs Dose MD= -17.0, P<0.001) but when control was compared with dose no significant difference was observed (MD= -3.25,P>0.05). Liver concentration of catalasewas altered significantly after stress and drug treatment (F=146.0, P<0.0001). Comparison between groups reveled that Mean concentration of catalase altered significantly except Control Vs Dose (MD= -2.25, P>0.05). Catalase change in kidney was significantly altered after stress and drug treatment (F=162, P<0.001). Comparison among groups has reveled that mean difference was significant in Control Vs Stress (MD= 29, P<0.001) Stress Vs Stress+Dose (MD= -26.5, P<0.001) and Stress Vs Dose (MD= -29.8,P<0.001). No significant difference was found in Control Vs Stress+Dose, Control Vs Dose and Stress+Dose Vs Dose). Muscles analyzed for catalase content exhibited significant difference (F=160, P<0.001). Comparison among groups was also significant at P<0.001 except Control Vs Dose. Concentration of catalase in heart muscle altered significantly after stress and *Cassia fistula* fruit pulp extract treatment (F=289, P<0.0001). Post

hoc analysis reveled that mean difference was significant among following groups; Control Vs Stress (MD= 36.3, P<0.001) Control Vs Dose (MD= -5.25, P<0.05) Stress Vs Stress+Dose (MD= -38.4, P< 0.001) and Stress Vs Dose (MD= -41.5, P<0.001). No significant difference was observed in Control Vs Stress+Dose (MD= -2.16,P>0.05) and Stress+Dose Vs Dose (MD= -3.09, P>0.05).

Glutathione Peroxidase (GPx):

The enzyme glutathione peroxidase, containing selenium as a prosthetic group, catalyzes the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids against oxidation by peroxides. Also act on reduced glutathione (GSH) and H_2O_2 to produce oxidized glutathione (GSSG) and H_2O; this enzyme can also use other peroxides as substrates. OH· and OH^- can be formed from H_2O_2 in a nonenzymatic reaction catalyzed by Fe_2^+ (the Fenton reaction,). O_2 and H_2O_2 are the substrates in the iron-catalyzed Haber-Weiss reaction (reaction that also produces OH· and OH–.

In the present study Glutathione peroxidase content was significantly decrease after stress treatment and this was further increased after the *Cassia fistula* fruit pulp extract treatment in all the tissues.

After stress treatment 40 percent decrease was noted in stressed animals brain as compared to control. This decrease was recovered after dose treatment in animals, which were subjected to stress and dose simultaneously. One way ANOVA reveled significant difference in Glutathione content in Brain (F=8.50, P<0.0002). Comparison among groups demonstrated that there is significant difference between Control Vs Stress (MD= 1.65, P<0.05) and Stress Vs Dose (F= -2.95, P<0.001). Remaining all the groups Control Vs Stress+Dose, Control Vs Dose, Stress Vs Stress+Dose and Stress+Dose Vs Dose did not exhibit significant difference. Stomach depicted comparatively lesser significant data in comparison to brain F value was 7.00 and P<0.0008. Post hoc analysis resulted in significant difference among following groups Control Vs Stress (MD= 3.2, P<0.001), Stress Vs Stress+Dose (MD= -2.0, P<0.05), Stress Vs Dose (MD= -2.0, P<0.05). Control Vs Sress+Dose (MD=1.2,P>0.05) Control Vs Dose (MD=1.2, P>0.05) and Stress+Dose Vs Dose resulted in non significant mean difference. Similar trend was observed for intestine. One way ANOVA analysis was significant (F=9.58,P<0.0001). But post hoc analysis for Control Vs Sress+Dose Control Vs Dose and Stress+Dose Vs Dose resulted in non significant mean difference although significant mean difference was observed for Control Vs Stress (MD= 1.85, P<0.01), Stress Vs Stress+Dose (MD= -1.5, P<0.05), Stress Vs Dose (MD= -2.35, P<0.001). Lungs depicted approximately 51.6 percent decrease in GPx content this was further returned to values comparable to control animals after treatment with drug. This change was significant (F=46.34,P<0.0001). Post hoc analysis resulted into significant mean difference in all the experimental groups except Control Vs Dose.

Reduced glutathione (GSH):

Reduced glutathione removes H_2O_2 in a reaction catalyzed by glutathione peroxidase, an enzyme that contains the selenium analogue of cysteine (selenocysteine) at the active site. This reaction is important, since accumulation of H_2O_2 may decrease the life span of the cell by causing oxidative damage to the cell membrane, leading to cell death.

Treatment with stress and dose significantly altered reduced gluthione levels in all the tissues. This was decreased by 33.34 percent in brain, 41.72 in in stomach, 43.23 in intestine, 15.78 in lungs, 47.32 in liver, 39.83 in kidney, 33.07 in muscle and in heart it was decreased by 41.93 percent. After dose treatment in brain (42.35%), stomach (58.02%), intestine (55.26%), Lungs (41.66%), liver (40.56%), kidney (47.29%), muscle (28.23) and in heart (52.65%) increase in reduced glutathione content was observed. One way ANOVA reveled significant difference in brain (F=19.28,P<0.0001), stomach (F=29.87,P<0.001), intestine (F=188.7,P<0.0001), lungs (F=9.51,P<0.001) liver (F=19.91,P<0.0001), kidney (F=18.60,P<39.83), muscle (F=19.98,P<0.008), heart (F=67.69,P<0.001).

Post hoc analysis of brain reveled significant mean difference in control Vs Stress (MD=2.15,P<0.001), Stress Vs Stress+Dose (MD= -1.8,P<0.001) and in Stress Vs Dose (MD= -1.65,P<0.001) and non significant mean difference among Control Vs Stress+Dose (MD= 0.35,P>0.05), Control Vs Dose (MD=0.05,P>0.05), and Stress+Dose Vs Dose (MD= -1.5,P>0.05).

In stomach significant mean difference in control Vs Stress (MD=2.9,P<0.001), Control Vs Dose (MD= 0.95,P<0.05), Stress Vs Stress+Dose (MD= -2.35,P<0.001) and in Stress Vs Dose (MD= -1.95,P<0.001) and non significant mean difference among Control Vs Stress+Dose (MD= 0.55,P>0.05), and Stress+Dose Vs Dose (MD= 0.40,P>0.05) was observed after Tukey's Multiple Comparison Test (Post hoc) test.

Comparison of groups with post hoc test for intestinal tissue reveled significant mean difference in all the groups (P<0.001) except Stress+Dose Vs Dose (MD= -0.15,P>0.05). Post hoc analysis for lungs depicted significance at two levels Stress Vs Stress+Dose (MD= -2.0) and Stress+Dose (MD= -1.3) were significant at P<0.001 and Control Vs Stress (MD=0.90) and Control Vs Stress+Dose (MD= -1.1)

were significant at P<0.05. Control Vs Dose (MD= -4.0) and Stress+Dose Vs Dose (MD= 0.70) were non significant. Post hoc analyis for liver exhibited significant difference among groups viz. Control Vs Stress (MD=4.8, P<0.001), Control Vs Stress+Dose (MD=2.65) and Stress+Dose Vs Dose (MD= -2.8) were significant at P<0.01; Stress Vs Stress+Dose (MD= -2.15) StressVs Dose (MD= -4.95) were having P value less then 0.05. Control Vs Dose (MD= -0.15) was found to be non significant. Tuckey's test for kidney depicted significance level P<0.01 for Control Vs Stress (MD=2.45), Stress Vs Dose (MD= -3.10). Stress Vs Stress+Dose (MD= -1.75,P<0.01) and Stress+Dose Vs Dose (MD= -1.35,P<0.05) were also found to be significant. Control Vs Stress+Dose and Control Vs Dose was non significant. Post hoc analysis for muscle was significant for all the groups except Control Vs Dose. Mean difference for heart exhibited significance between P<0.001 and P<0.05 for all the groups.

Malondialdehyde (MDA):

Lipid peroxidation has often been regarded simply as an undesirable side reaction, but it is also a normal part of metabolism. Lipid radicals also readily react with oxygen, with the subsequent process, termed lipid peroxidation, producing damage to the membranes and enzymes. The resulting lipid peroxyl radicals decompose to aldehydes, the most abundant being malondialdehyde and 4-hydroxy-2,3-nonenal.

After treatment with stress and *Cassia fistula* fruit pulp powder malondialdehyde content was increased in all the tissues analyzed. One way ANOVA depicted that increase was significant at P<0.0001 in all the tissues. Post hoc analysis for brain exhibited significant mean difference when analysis was performed among groups. Only Control Vs Dose was found to be non significant. When same test was applied for stomach all the comparisons among the groups resulted into significant mean difference (P<0.001). Intestinal tissue was affected in the same manner as stomach but significance level varied between P<0.001 and 0.05. Stress+Dose Vs Dose was not significant for intestine. Post hoc for other tissues namely lungs, kidney, liver and muscle was not much promising in all the tissues comparison between Control Vs Stress+Dose, Control Vs Dose and Stress+Dose Vs Dose was non significant. Rest all the comparisons between Control Vs Stress, Stress Vs Stress+Dose and Stress Vs Dose were significant at P<0.001. Heart followed the same pattern as it was for intestine. Significant mean diffrence was found between Control Vs Stress (P<0.001), Control Vs Dose (P<0.01), Control Vs Stress+Dose (P<0.01), Stress Vs Stress+Dose (P<0.001), Stress Vs Dose (P<0.001) but found to be non significant for Stress+Dose Vs Dose (P>0.05).

DISCUSSION

In present study, a significant decrease in SOD, CAT, GPX and reduced GSH activity was observed in all tissues of mice subjected to combination of stress as compared to control. A significant increase in SOD, CAT, GPx, and reduced glutathione levels were observed in all the tissues of stressed animals fed with pulp powder extract. In tissues of stressed mice, a significant increase in MDA level was observed which was significantly inhibited after feeding pulp powder extract. Highly significant increase in SOD activity was noticed in lung, liver, kidney, lungs and muscle; CAT activity was observed in all the tissues except kidney. Increase in GPx and GSH was not much promising after post hoc analysis. Measurement of Lipid peroxidantion was found to be more significant in brain, liver and intestine as compared to other tissues analyzed. This suggests that as leaves, bark or flowers[6,7,24,25], pulp of C. *fistula* pod also has potent antioxidant power. Acute or chronic stress negatively alters antioxidant status of body tissues. Excess release of glucocorticoids during severe stress is related with release of free radicals this ultimately alters endogenous antioxidant status of body tissues including brain, liver, muscle, kidney etc., [13, 14]. This in vitro and vivo antioxidant activity has been of the *C. fistula* has been further supported by other workers who reported dose dependent protective effects of aqueous and methanolic extract of the *C. fistula* bark against lipid per oxidation and free radical generation in liver and kidney homogenates [6]. Amongst the vegetative organs of *C. fistula*, bark has the highest antioxidant potential followed by the old leaves, young leaves and the twigs [9, 24]. Both phenolic and flavonoid components are reported to be associated with antioxidative action in biological system as they scavenge singlet oxygen and free radicals[26, 27]. Polyphenolic contents have been reported to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products [28]. Free radicals formed in biological systems have been implicated as a highly damaging species, capable of damaging almost every molecule found in living cells. These radicals have capacity to join nucleotides in DNA and cause strand breakage; in addition they are initiators of lipid per oxidation process, abstracting hydrogen atoms from fatty acids in biological membranes. Per oxidation inhibiting activity has been reported in various solvent extracts of different parts of *C. fistula* [24].

Table-2: Superoxide dismutase (SOD) activity in various tissues of control and experimental animals.

Group	Treatment	Super oxide dismutase (SOD) (Percentage inhibition of NBT reduction ± SD)							
		Brain	Stomach	Intestine	Lungs	Liver	Kidney	Muscle	Heart
I.	Control	76.66±6.41	72.77±16.04	72.21±26.94	86.33±0.39	95.27±0.32	87.22±3.21	81.11±1.28	76.66±3.85
II.	Stress	17.77±3.85^{+++}	16.11±5.77^{+++}	17.22±4.49^{++}	20.55±1.92^{+++}	27.77±3.85^{+++}	30.00±16.67^{+++}	30.44±1.79^{+++}	24.99±4.49^{+++}
III.	Stress+Dose	56.66±19.24^{++}	40.55±7.05^{++}	46.11±12.18^{++}	56.66±6.41^{+++}	81.11±6.42^{+++}	72.78±8.34^{+++}	56.66±6.41^{+++}	66.11±10.90^{+++}
IV.	Only Dose	80.56±16.03^{+++}	60.55±5.77^{+++}	57.22±22.45^{+}	68.89±3.84^{+++}	91.11±3.84^{+++}	64.44±2.57^{++}	67.22±1.93^{+++}	72.77±7.05^{+++}

All values are represented as Mean ± SD (n = 10)
P value:
$^{+}$ < 0.001;
$^{++}$ < 0.01;
$^{+++}$ < 0.05 When compared with control untreated animals.

Table-3: Catalase (CAT) activity in various tissues of control and experimental animals.

Group	Treatment	Catalase (μ Moles of H_2O_2 utilized /min./mg of protein ± SD)							
		Brain	Stomach	Intestine	Lungs	Liver	Kidney	Muscle	Heart
I.	Control	48.50±4.04	57.75±4.33	45.00±3.46	58.75±4.33	66.25±6.06	48.50±4.04	62.00±4.62	54.50±4.04
II.	Stress	19.50±1.73^{+++}	17.25±1.44^{+++}	15.00±1.15^{+++}	28.25±2.60^{+++}	28.25±2.60^{+++}	19.50±1.73^{+++}	25.75±2.02^{+++}	18.25±1.44^{+++}
III.	Stress+Dose	43.50±2.89^{+++}	40.50±2.89^{+++}	42.75±3.18^{+++}	45.00±3.46^{+++}	51.25±3.75^{+++}	46.00±3.46^{+++}	42.75±3.17^{+++}	56.66±3.17^{+++}
IV.	Only Dose	57.75±4.33^{+++}	56.75±4.33^{+++}	51.25±3.75^{+++}	62.00±4.62^{+++}	68.50±5.20^{+++}	49.25±3.75^{+++}	66.25±6.02^{+++}	59.75±4.33^{+++}

All values are represented as Mean ± SD (n = 10)
P value:
$^{+}$ < 0.001;
$^{++}$ < 0.01;
$^{+++}$ < 0.05 When compared with control untreated animals.

Table 4: Glutathione peroxidase (GPx) activity in various tissues of control and experimental animals.

Group	Treatment	Glutathione peroxidase (GPX) (Moles of GPx utilized /mg protein/min ± SD)							
		Brain	Stomach	Intestine	Lungs	Liver	Kidney	Muscle	Heart
I.	Control	4.05±0.87	4.65±2.48	4.25±0.87	4.45±0.52	8.10±1.62	4.65±0.40	4.65±0.40	5.35±1.67
II.	Stress	2.40±0.35^{+}	1.45±0.40^{+}	2.40±0.69^{+}	2.15±0.63^{++}	3.40±0.11^{++}	1.80±0.46^{+++}	2.70±0.69^{++}	2.00±0.23^{++}
III.	Stress+Dose	3.95±1.10^{+}	3.45±1.33^{+}	3.90±0.92^{+}	3.65±0.63^{+}	4.35±0.40^{++}	5.05±1.79^{+}	3.60±0.23^{+}	2.80±0.11^{+++}
IV.	Only Dose	5.35±2.02^{+}	3.45±0.98^{++}	4.75±1.33^{+}	5.05±0.40^{+++}	6.80±1.50^{++}	4.35±0.17^{+++}	4.60±0.11^{++}	4.10±0.35^{+++}

All values are represented as Mean ± SD (n = 10)
P value:
$^{+}$ < 0.001;
$^{++}$ < 0.01;
$^{+++}$ < 0.05 When compared with control untreated animals.

Table 5: Reduced glutathione (GSH) in various tissues of control and experimental animals.

Group	Treatment	Reduced Glutathione (GSH) (GSH mg/g of tissue ± SD)							
		Brain	**Stomach**	**Intestine**	**Lungs**	**Liver**	**Kidney**	**Muscle**	**Heart**
I.	Control	6.40±0.46	6.95±0.17	6.70±0.35	5.70±0.46	10.10±2.31	6.15±1.21	6.35±0.75	6.90±0.81
II.	Stress	4.25±0.29^{+++}	4.05±0.87^{+++}	3.80±0.35^{+++}	4.80±0.35^{+}	5.30±0.35^{++}	3.70±0.35^{++}	4.25±0.87^{+}	3.80±0.23^{+++}
III.	Stress+Dose	6.05±0.87^{++}	6.40±0.81^{++}	5.90±0.23^{+++}	6.80±1.50^{+}	7.45±0.75^{++}	5.45±1.10^{+}	5.45±0.29^{+}	5.85±0.40^{+++}
IV.	Only Dose	5.90±0.81^{++}	6.00±0.69^{+}	6.05±0.06^{+++}	6.10±0.23^{+++}	10.25±2.02^{++}	6.80±0.81^{+++}	6.30±0.58^{++}	5.15±0.17^{+++}

All values are represented as Mean ± SD (n = 10)

P value:

$^{+}$ < 0.001;

$^{++}$ < 0.01;

$^{+++}$ < 0.05 When compared with control untreated animals.

Table 6: Malanoldialdehyde (MDA) in various tissues of control and experimental animals.

Group	Treatment	Malondialdehyde (MDA) (n moles /ml ± SD)							
		Brain	**Stomach**	**Intestine**	**Lungs**	**Liver**	**Kidney**	**Muscle**	**Heart**
I.	Control	1.35±0.01	0.79±0.29	0.91±0.37	1.95±0.64	2.50±0.30	1.74±0.74	1.47±0.90	1.54±0.17
II.	Stress	3.13±0.46^{+++}	4.07±0.21^{+++}	3.89±1.03^{++}	4.14±1.18^{+}	4.91±0.88^{++}	4.25±1.33^{+}	4.04±0.85^{++}	3.31±0.66^{++}
III.	Stress+Dose	2.45±0.29^{+}	2.44±0.37^{+++}	2.17±0.26^{+}	2.65±0.05^{+}	2.59±0.61^{++}	1.98±0.63^{+}	2.31±0.46^{+}	2.33±0.32^{+}
IV.	Only Dose	1.21±0.02^{+++}	1.58±0.18^{+++}	1.70±0.25^{++}	2.45±0.47^{+}	1.99±0.62^{++}	2.04±0.29^{+}	2.23±0.15^{++}	2.29±0.31^{+}

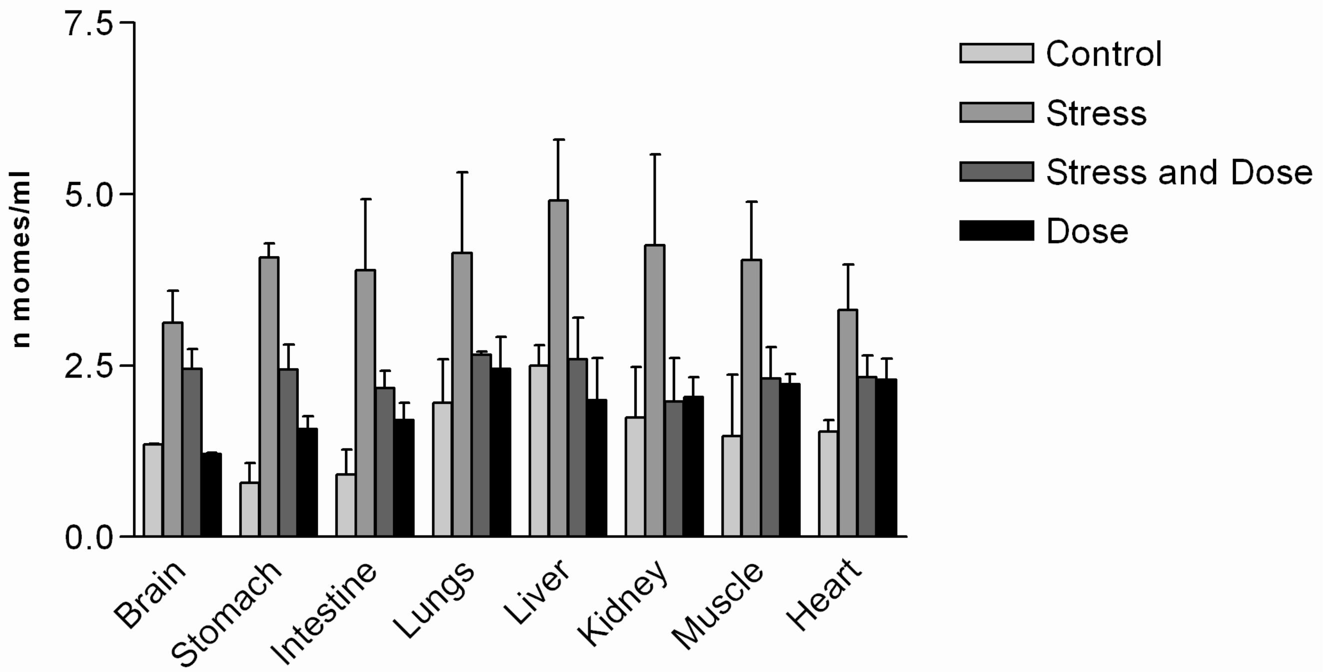
All values are represented as Mean ± SD (n = 10)

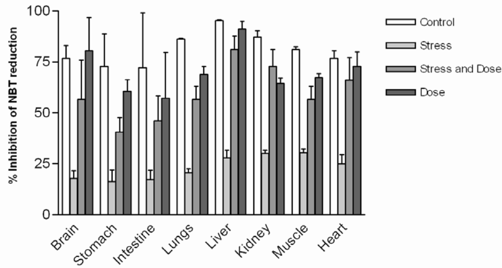
P value:

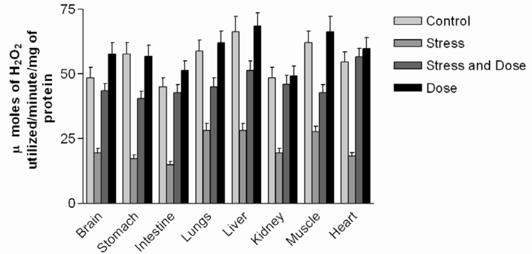
$^{+}$ < 0.001;

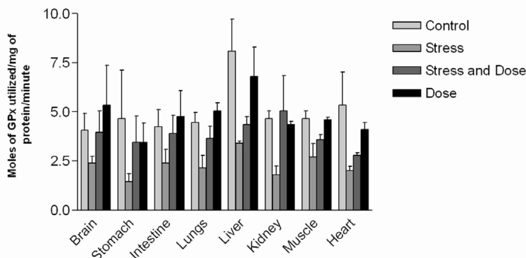
$^{++}$ < 0.01;

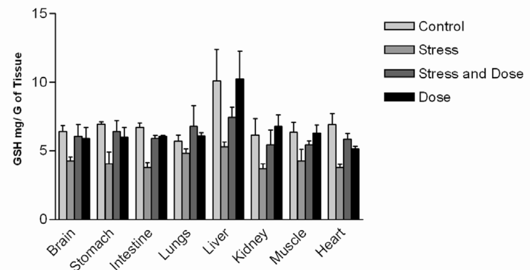
$^{+++}$ < 0.05 When compared with control untreated animals.


Malanoldialdehyde
n momes/ml
7.5
5.0
2.5
0.0
Control
Stress
Stress and Dose
Dose
Brain
Stomach
Intestine
Lungs
Liver
Kidney
Muscle
Heart


Superoxide Dismutase
% Inhibition of NBT reduction
Control
Stress
Stress and Dose
Dose
Brain
Stomach
Intestine
Lungs
Liver
Kidney
Muscle
Heart


Catalse
µ moles of H_2O_2 utilized/minute/mg of protein
Control
Stress
Stress and Dose
Dose
Brain
Stomach
Intestine
Lungs
Liver
Kidney
Muscle
Heart


Gluthione Peroxidase
Moles of GPx utilized/mg of protein/minute
Control
Stress
Stress and Dose
Dose
Brain
Stomach
Intestine
Lungs
Liver
Kidney
Muscle
Heart


Reduced Glutathione
GSH mg/ G of Tissue
Control
Stress
Stress and Dose
Dose
Brain
Stomach
Intestine
Lungs
Liver
Kidney
Muscle
Heart

Over the past few years there has been exponential growth in the number of reports indicating that excessive free radical production and lipid per oxidation are actively involved in the pathogenesis of a wide number of diseases. Consequently there has been a growing interest in potential health promoting properties of phytochemicals of plant origin. Special attention has been given to phenolic derivatives[29-31].

The results of present study with *Cassia fistula* bark extracts have good correlations with the pharmacological property of *Cassia fistula* pulp powder as antioxidant. Plants which belong to Caesalpinaceae family are rich in flavonoids and bio flavonoids are known for their antioxidant activities. Further studies for elucidation of antioxidant effects of active compound (s) present in the extract is under progress.

REFERENCES

1. Deshpande DJ. *A handbook of Herbal remedies*. Agrobios Publications, Jodhpur, pp24–26,2008.
2. Bhakta T, Mukherjee PK, Pal M, Saha BP. *Studies on antitussive activity of Cassia fistula (Leguminosae) leaf extract*. Pharm Biol, **36**: 140–143, 1998.
3. Bhakta T, Mukherjee PK, Pal M, Saha BP, Studies on *in vivo* wound healing activity of *Cassia fistula* Linn. leaves (Leguminosae) in rats. *Nat Prod Sci*, **4**: 84–87,1998.
4. Gupta M, Mazumdar UK, Rath N, Mukhopadhyay DK, Antitumour activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. *J Ethnopharmacol*, **72**:151–156, 2000.
5. Ali MA, Sayeed MA, Bhuiyan MSA, Sohel FI, Antimicrobial screening of *Cassia fistula* and *Mesua ferrea*. *J Med Sci*, **4**: 24–29, 2004.
6. Ilavarasan R, Mallika M, Venkataraman S, *Anti-inflammatory and antioxidant activities of Cassia fistula bark extracts Afr J Trad CAM*, **2**: 70–85, 2005.
7. Manonmani G, Bhavapriya V, Kalpana S, Govindsamy S, Apparanantham,T, Antioxidant activity of *Cassia fistula* (Linn) flowers in alloxan induced diabatic rats. *J. Ethanopharmacol*, **97**:39–42, 2005.
8. Yen GC, Duh PD, Chuang DY, Antioxidant activity of anthraquinones and anthrone. *Food Chem*, **70**: 437–441, 2000.
9. Yadav RN, Verma VA, New biologically active flavone glycoside the seed of *Cassia fistula* Linn. Has been identified. *J Asian Nat Prod Res*, **5**: 57–61, 2003.
10. Kuo YH, Lee PH, Wein YS, Four new compounds from the seeds of *Cassia fistula*. *J. Nat. Prod*, **65**: 1165–1167, 2002.
11. Patil AD, Deshpandey VH, A new dimeric proanthocyanidin from *Cassia fistula* sapwood. *Ind J Chem*, **21B**: 626–628, 1982.
12. Bahorun T, Vidushi S, Neergheen P, Okezie I, Aruoma OI, Phytochemical constituents of *Cassia fistula*. *Afr J Biotech*, **4**:1530–1540, 2005.
13. Dutta A, De B, Seasonal variation in the content of sinnoside and rhein in leaves and pods of *Cassia fistula*. *Ind J. Pharm Sci*, **60**: 388–390, 1998.
14. Aruyoma OI, Free radicals, oxidative stress and antioxidants in human health and disease. *J Amer Oil Chemist's Soc*, **75**:199–211,1998.
15. Anderson D, Antioxidant defences against reactive oxygen species causing genetic and other damage. *Mutation Res*, **350**:103–108, 1999.
16. Tanner H, Brunner HR, *Gentranke-Analytik*. Germany: Verlag Heller Chemie und Verwaltunsgesellschaft mbh., pp 206, 1979.
17. Zhishen, J, Mengcheng T, Jianming W, The determination of flavonoid contents of mulberry and their scavenging effects on superoxide radicals. *Food Chem*, **64**: 555–559, 1999.
18. Benzie IF, Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. *Anal Biochem* **239**: 70–76, 1996.
19. Winterbourn CC, Hawkins RE, Brian M, Carrell RW, The estimation of red cell superoxide dismutase activity. *J Lab Clin Med*, **85**: 337–341, 1975.
20. Sinha AK, Colorimetric assay of catalase. *Anal. Biochem*, **47**: 389, 1972.
21. Paglia DE, Valentine WN, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxides. *J lab clin Med*, **70**:155–169, 1976.
22. Beutler E, Glutathione in red blood cell metabolism. *A manual of biochemical Methods*. NewYork, Grund and Strattun, pp112–114, 1975.
23. Buege JA, Aust SD, The thiobarbituric acid assay. *Meth Enzymol*, **52**: 306–307, 1978.
24. Siddhuraju P, Mohan PS, Becker K, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem*, **79**: 61–67, 2000.
25. Bhavapriya V, Kalpana S, Govindasamy S, Apparanantham T, Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetic rats. *J Ethnopharmacol*, **97**:39–42, 2005.
26. Galati G, O'Brian PJ, Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. *Free Rad Biol Med*, **37**: 287–303, 2004.
27. Rice-Evans G, Sampson J, Bramley PM, Holloway DE, Why do we expect carotenoids to be antioxiudants in vivo. *Free Radic Res*, **26**:381–398, 1997.
28. Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO, Skibsted H, Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Radical. Res*, **30**:207–220, 1999.
29. Manian R, Nagarajan A, Sidduraju P, Manian S, The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L) O. Kunz, Ficus bengalensis L. and *Ficus racemosa* L. *Food Chem*, **107**:1000–1007, 2008.
30. Luximno-Ramma A, Bahourn T, Crozier A, Zbarsky V, et al. *Characterization of the antioxidant functions of flavonoids and proanthocyanidines in Maritian black J Agri Food Chem*, **38**: 357–367, 2005.
31. Adhami VM, Malik A, Zaman N, Sarafraz S, Siddiqui IA, Syed DN, Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vitro* and *in vivo*. *Cancer Res.*, **13**:1611–1619, 2007.

Anti-Inflammatory Activity of Leaves of *Argyreia Nervosa* in Carrageenan-Induced Paw Edema in Rats

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ABSTRACT

The present study was designed to investigate the anti-inflammatory activity of the water extract of *Argyreia nervosa*. Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Therefore the search for a better tolerated anti-inflammatory agent appears to be a necessity. *Argyreia nervosa* is used as a folk medicine for the treatment of inflammation in India. Present study revealed that the plant *Argyreia nervosa* possesses a significant anti-inflammatory activity as evidencesd in carrageenan induced paw edema method, which supports the folkloric claim of the anti-inflammatory activity of the plant. Our finding supports the reported therapeutic use of herb *Argyreia nervosa* in tribal medicine for the treatment of inflammation.

Keywords: Inflammatory disease, Argyreia nervosa, herbal drugs..

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INTRODUCTION

The plant kingdom is abundant in species that act as anti-inflammatory to animal tissue. Whilst they are rarely as immediately powerful as the steroid drugs, they are very rarely as dangerous and potentially life threatening. In a well known study, 163 species of plants and fungi were tested to determine their anti-inflammatory activity. Due to adverse effects associated with the use of NSAIDs and other medications, there arise new scopes for Herbs and Herbal Formulation in treatment of inflammatory diseases.[1]

Argyreia speciosa (Linn.f.) sweet belongs to family Convolvulaceae is a climbing shrub with woody tomentose stem, found mainly in Deccan, Karntaka and East slopes of the West Ghats at an altitude of 900m.[2] It is commonly known as Elephant creeper and in Samudra-sok Hindi.[3]Traditinally, leaves are used by Rajasthani tribes to prevent conception.[4] Seeds of *Argyreia nervosa* found to possess hypotension, spamolytic,[5] and anti-inflammatory activity.[6] Chemical analysis revealed the presence of triterpenoids, flavanoids, steroids and lipids.[7] Roots of Argyreia nervosa proved the immunomodulatory activity against the myelosuppressive effects induced by Cyclophosphamide.[8] 24R-ergost-5- en-11-oxo-3 beta-ol alpha –D glucopyranoside xylose was isolated from seeds of *Argyreia nervosa* known as Argyreioside.[9]

CLASSIFICATION [10]

Kingdom	: Plantae – Plants
Subkingdom	: Tracheobionta – Vascular plants
Superdivision	: Spermatophyta – Seed plants
Division	: Magnoliophyta – Flowering plants
Class	: Magnoliopsida – Dicotyledons
Subclass	: Asteridae
Order	: Solanales
Family	: Convolvulaceae.
Genus	: Argyreia Lour. – *Argyreia*
Species	: *Argyreia nervosa* (Burm. f.) Bojer.

Figure 1: Leaves

Botanical description: (See on Figure: 1)

A very large climber; stem stout, white-tomentose. Leaves are 7.5-30.0 cm. in diameter, acute, ovate, glabrous above, persistently white-tomentose beneath, base cordate; petioles 5-15 cm. long, white-tomentose. Flowers in subcapitate cymes; penduncles 7.5-15 cm. long, stout, white-tomentose; bracts large, ovate-lanceolate with a long acumen, thin, veined, pubescent outside, glabrous inside, deciduous the outer sometimes 5 cm. long; pedicels very short often almost 0, white-tomentose.Calyx white-tomentose outside; corolla 5-6.3 cm. long, tubular-infundibuliform, the bands silky pubescent outside, tube somewhat inflated, white pubescent outside, rose purple and glabrous inside. Ovary glabrous. Fruit glabrous, 2.0 cm. in diameter, apiculate.[4]

MATERIALS AND METHODS

Plant material

The fresh leaves of *Argyreia nervosa* were collected in the months of July-August from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amaravati. A voucher specimen was submitted at Institute's herbarium department for future reference. Dried leaves were ground to coarse powder.

Extraction

For extraction coarsely and air dried 350gms powder of *Argyreia nervosa* leaves was taken. Extraction was carried out by using Distilled water by a maceration process, the extract was concentrated to dryness and it was preserved in a refrigerator. The maceration process was carried out until the solvent found to be colorless. Finally the solvent was filtered and distilled off. By using rotary vacuum flask evaporator.

Animals

Albino wistar rats of either sex weighing 100-150g were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20°C; relative humidity 60 - 70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by institutional animal ethical committee (Registration No.751/01/abc/CPCSEA).

In vivo Anti-inflammatory activity

Carrageenan-induced paw edema in rats

Albino wistar rats of either sex weighing 100-150g were divided in to four groups as shown in table. 1.

The animals were divided into groups as control, Standard, Test-1 and Test-2, 6 animals in each group. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats, before topical application of the formulation. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0, 30, 60, 90, 120, 180 min. after the carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage anti-inflammatory activity was calculated. % inhibition of paw edema is calculated by comparing the control.[11]

The average foot swelling in test as well as standard groups was compared with that of the control group and the % edema was calculated by using the formula.

$$\% \text{ Edema} = \frac{Co - Cr}{Co} \times 100$$

Where, C_r = Average paw volume of treated group.
C_o = Average paw volume of control group.

Statistical Analysis

The values obtained were expressed as mean ± S.E.M. Statistical significance of the differences between control and treated groups was calculated by one way ANOVA

Table 1: Group specification and dose for individual treatment.

Group no.	Group Specification
I	Control (Ointment base)
II	Standard (Containing 1.16% Diclofenac sodium)
III	Formulation 1 (Containing 3% water extract of Argyreia nervosa)
IV	Formulation 2 (Containing 5% water extract of Argyreia nervosa)

Table 2: Effect of Standard on carrageenan-induced paw edema in rat.

Animal no.	Initial Paw Volume (ml)	Paw Volume after 3 hr.	Difference in paw volume (Edema)
1	0.066	0.15	0.084
2	0.065	0.16	0.098
3	0.066	0.16	0.094
4	0.064	0.15	0.086
5	0.065	0.13	0.065
6	0.065	0.14	0.075
Mean ± SEM	0.0651±0.0006	0.1483±0.011	0.0831±0.0110

Table 3: Effect of Formulation 1 on carrageenan-induced paw edema in rat.

Animal no.	Initial Paw Volume (ml)	Paw Volume after 3 hr.	Difference in paw volume (Edema)
1	0.066	0.26	0.194
2	0.067	0.25	0.183
3	0.066	0.25	0.184
4	0.065	0.26	0.195
5	0.067	0.25	0.183
6	0.066	0.26	0.194
Mean ± SEM	0.06616±0.00071	0.255±0.0054	0.1888±0.0060

Table 4: Effect of Formulation 2 on carrageenan-induced paw edema in rat.

Animal no.	Initial Paw Volume (ml)	Paw Volume after 3 hr.	Difference in paw volume (Edema)
1	0.062	0.23	0.168
2	0.063	0.24	0.177
3	0.065	0.24	0.175
4	0.064	0.22	0.156
5	0.063	0.24	0.177
6	0.063	0.24	0.177
Mean ± SEM	0.06333±0.0010	0.235±0.0083	0.1716±0.00843

Table 5: Effect of various treatments on carrageenan-induced paw edema in rats.

Treatment	Initial Paw Volume (ml)	Paw Volume after 3 hr. (ml)	Increase in Paw Volume (ml)	% inhibition
Control	0.0646±0.00054	0.621±0.00661	0.556±0.0077	-
Standard	0.0651±0.0006	0.1483±0.011	0.0831±0.0110	83.97
Formulation 1	0.06616±0.00071	0.255±0.0054	0.1888±0.0060	66.04
Formulation 2	0.06333±0.0010	0.235±0.0083	0.1716±0.00843	69.13

followed by Dunnett's test. $P<0.05$ was considered to be significant.

RESULT:

In-vivo Anti-inflammatory activity

1. In-vivo Anti-inflammatory activity of Formulation 1 was given after the injection of carrageenan (1%, 0.1 ml) into sub-planter region of hind paw of rat produced edema (0.255±0.0054 ml) when compared with the mean of initial paw volume (0.06616±0.00071 ml) and the difference observed was 0.1888±0.0060 ml (Table- 03). The inhibition of paw edema was found to be 66.04% (Table-05).

2. In-vivo Anti-inflammatory activity of Formulation 2 was given after the injection of carrageenan (1%, 0.1

ml) into sub-planter region of hind paw of rat produced edema (0.235± 0.083 ml) when compared with the mean of initial paw volume (0.06333 ± 0.0010 ml) and the difference observed was 0.1766 ± 0.0843 ml (Table- 04) The inhibition of paw edema was found to be 69.13 % (Table-05).

DISCUSSION

Formulations exhibited significant anti- inflammatory activity against carrageenan-induced rat paw edema. Formulation 1 and 2 inhibited the edema formation significantly induced by carrageenan to an extent of 66.04 and 69.13% respectively. Diclofenac sodium as a reference standard inhibited the edema formation due to carrageenan to an extent of 83.97 % at the topically applied 0.5 gm (Table-05). Diclofenac sodium and both the formulations of water extract of *Argyreia nervosa* leaves exhibited significant anti-inflammatory activity against carrageenan-induced rat paw edema. The edema formation was greatly inhibited between 2-3 hours after sub-planter injection of carrageenan in all treated groups. (Table-05).

CONCLUSION

As per previous reports, phytochemical analysis of *Argyreia nervosa* confirms the authenticity of the plants. The present study of *Argyreia nervosa* leaves might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs. In present study *Argyreia nervosa* was taken to evaluate in vivo anti-inflammatory activity. Present study revealed that the plant *Argyreia nervosa* possesses a significant anti-inflammatory activity in carrageenan induced paw edema method, which supports the folkloric claim of the plant.

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REFERENCES

1. Derle DV, Gujar KN, Sagar BSH. Adverse effects associated with the use of non-steroidal anti-inflammatory drugs: An overview. *Indian J. Pharm. Sci.* **68**(4): 409–41.
2. Gamble JS (1956). *Flora of Madras*. Botanical survey of India, Calcutta, **2**: 556, 2006.
3. Warrier PK, Nambiar VPK and Ramankutty C. Indian Medicinal Plants of India. Orient Longman, Chennai, **I**: 191–193, 1997.
4. The Wealth of India, A dictionary of Indian Raw materials and industrial products. *Publication and Information Directorate*, CSIR, New Delhi, India, **I-A**: 87–88, 2004.
5. Agarwal SR and Rastogi RP. Pharmacognostical and Preliminary Phytochemical Studies of *Argyreia nervosa* Burm. *Indian Journal of Pharmacology*, Sept **35**: 118–119, 1974.
6. Gokhale AB, Damre AS, Kulkarni KR. and Saraf MN. Preliminary evaluation of anti-inflammatory and antiarthritic activity of S.lappa, A.speciosa and A.aspera. *Phtyomedicine*. July **9**(5):433–437, 2002.
7. Srivatasav A, Shukla Tau SP, Kumar S. *Journal of Aromatic and Medicinal Plants* **20**(3): 774–778, 1998.
8. Gokhale AB, Damre AS, Kulkarni KR. and Saraf MN. Investigation in to the Immunomodulatory activity of Argyreia speciosa. *Journal of Ethnopharmacolgy*, Jan **84**(1): 109–114, 2003.
9. Rahman Ali, Ali.M and Khan WZ. *Pharmacognostical and Preliminary Phytochemical Studies of Argyreia nervosa Burm Pharmazie*, Jan **58** (1): 60–62, 2003
10. http://plants.usda.gov, Accessed at 2/11/09.
11. Vogel HG. Drug Discovery and Evaluation Phrmacological Assays. Springer Publications, *New York*: 692–769, 2002.

Botanical description: (See on Figure: 1)

Pharmacognosy of a local market sample of Parpataka *Mollugo cerviana* (L.) Ser.

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ABSTRACT

Parpataka is an important Ayurvedic drug used for the treatment of fevers particularly. The local market sample of Parpataka is identified as *Mollugo cerviana* (L.) Ser. The botanical, macroscopical, microscopical characters of root, stem, leaf, whole plant macerate, powder analysis, histochemical tests, physical constants and fluorescence studies are presented.

Keywords: *Mollugo cerviana*, macroscopical, microscopical characters, powder microscopy studies, physical constants, fluorescence studies.

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INTRODUCTION

The identity and pharmacognosy of a locally sold crude drug in the name of Parpataka has been shown as *Mollugo cerviana* (L.) Ser. and the distinguishing characters of the same with *Fumaria indica* (Hassk.) pug.

Parpataka is an important Ayurvedic drug used in the Indian systems of medicine. The whole plant possesses medicinal properties[1-2]. The drug is diuretic, anthelmintic, digestive and relieves constipation. It is bitter, cooling and constrictor. It is used in the treatment of Rakta pitta (haemorhage), Trishna (Thirst), Brama (giddiness) and Daaha (burning sensation)[3]. In spite of its manifold uses, the drug remains controversy because several plants are used and sold under the name Parpataka in different parts of the country.

Some of the plants used as Parpataka are *Fumaria indica* and its allied sps, *Polycarpaea corymbosa* (L.) Lam., *Glinus oppositifolius* (L.) A.DC., *Mollugo nudicaulis* Lam., *Hedyotis corymbosa* (L.) Lam. and its allied species, *Glossocardia bosvallea* (L.f.) DC. and *Rungia* repens (L.) Nees [4-5].

During a market survey of crude drugs, it was observed that a drug locally known as Parpatakamu (Telugu) was used as Parpataka in the Ayurveda and in the Siddha preparations of South India. On a critical study, it was found that this plant was a totally different from the above mentioned taxa. Hence, it became necessary to identify this market sample of the drug botanically. Further, since the botanically identified plant *M. cerviana* has been accepted by a majority of Ayurvedic physicians as the true Parpataka as mentioned in the Ayurvedic classics, the distinguishing characters between *Mollugo cerviana* and *F. indica* was thought necessary and they are also presented.

Samples of *M. cerviana* known as Parpatakamu crude drug were obtained from the crude drug dealers of Tirupati market and also from the crude drug stores of the Srinivasa Ayurvedic Pharmacy, Tirupati, where this is used as Parpataka in Ayurvedic preparations.

Identification

Since the drug Parpatakamu happens to be a regional name, a literary survey was made to find out if the name has been used for other botanically identified plants. A plant under the name *Mollugo cerviana* and in tamil called as Parpadagam [6]. A check with the local Vaidhyas revealed that the same plant is known as Parpatakamu in Telugu also. This gave a clue on its identification. Hence, it was concluded that the market sample of the drug under the name Parpadagam which has been used as Parpataka, belongs to family Molluginaceae. In siddha

system whole plant promotes antipyretic and astringent activity.[7]

The Herbarium specimen was collected by author (205) on 14th November 2006 in the Panapakam Forest guest House area, Chittoor district, Andhra Pradesh and it is authenticated[8] and deposited in the Herbarium of the Sri Venkateswara University, Tirupati, Andhra Pradesh.

MATERIALS AND METHODS

The plant material was collected from Tirupati, Andhra Pradesh. The voucher herbarium specimen was processed followed by standard procedures[9], macro- and microscopical studies[10-11], phytochemical studies, fluorescence analysis and powder analysis were carried out [12-15].

Taxonomy

Mollugo cerviana (L.) Ser. in D.C Prodr.1 : 392; 1824, Wight and Arn. Prodr. Fl. Ind. Orient. 44, 1834; Hook. f. Fl. Brit. India 2, 663, 1879; Gamble, Fl. Madras 1 : 553 (390), 1919; Backer in Steenis, Fl. Males. I. 4 : 268, 1951; Matthew, mat. Fl. Tamilnadu Carnatic 225, 1981; Var. *Spathulifolia fenzl*, Ann. Wiener Mus. Naturgesh.1 : 379, 1836; *Pharmaceum cerviana* L. Sp. Pl. 272 753.

External Morphology

Annual glabrous herb grows up to 12 cm tall, branchlets glacous, in whorls of 8 from the root stock. Radical leaves rosulate, narrow 0.5-1 cm long, cauline leaves 5-7 in a whorl at nodes, narrowly linear or acicular, 1-1.5 cm long 0.2 cm, width, sessile or subsessile, glacous below, base attenuate, margin entire, apex obtuse, petiole upto 1 cm. Flowers white in terminal and axillary polychasial umbellate cymes. Peduncle slender 1.5 cm, bract subulate 0.5 mm, pedicel upto 5 mm. Flowers 2.5 mm across, sepals 5, subequal, elliptic, 1-5.2 mm, obtuse. Stamens 5-8, filaments 1 mm. Ovary 3-lobed, styles 3, upto 0.2 mm (Plate1).

RESULTS

Root

Macroscopical Characters

Roots are long, lateral roots few, outer zone is not peelable, slightly bitter to taste.

Plate 1: *Mollugo cerviana* (L.) ser. Entire plant
Pharmacognosy of a local market sample of Parpataka

Microscopical Characters

Transverse section of the thin root is 350 µm in diameter and has no growth rings. Transverse section of the thick root is 800 µm in diameter and has a distinct growth rings. The epidermis of the root is broken and disintegrated. The cortex is narrow comprising of 4-5 layers of Parenchyma cells. Secondary xylem is solid, dense and uniformly circular. It consists of wide, thick walled vessels. In thicker roots, distinct growth ring and semiringporous vessels are present. The xylem fibres are thick walled and lignified. The vessels include both narrow (15 µm) and wide elements (30 µm). Secondary phloem is made up of 4 layers of cells (Fig. 1.1-3).

Stem

Macroscopical Characters

Stems are filiform very slender, terete, brown coloured and intermodes are elongated, branchlets glacous, in whorls of 7-8 from the root stock. Slightly bitter to taste, no specific smell, stem is not peelable, with 0.5 mm thickness.

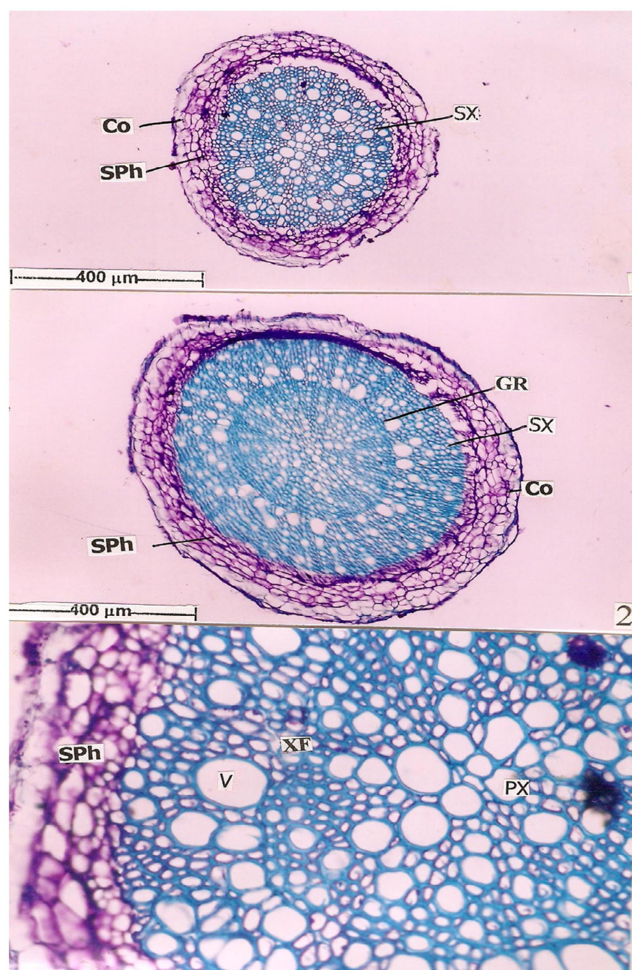

Figure 1: Anatomy of the root
1.1 T.S. of young root entire view
1.2 T.S. of old root entire view
1.3 T.S. of young root a sector enlarged

Microscopical Characters

Transverse section of the stem is 420 µm in diameter, consists of a single layer of spindle shaped, thick walled epidermal cells with thick cuticle. A single layer of hypodermal cells are present beneath the epidermis, these cells are similar with epidermal cells. The epidermal and hypodermal layers are 10-15 µm thick. The cortex is made up of 4-5 layers of sclerenchymatous cells with highly thickened lignified walls measuring about 40 µm thick.

The vascular bundles are arranged in the form of a ring around pith. It comprising of a ring of large, compact, metaxylem elements and one or two protoxylem elements at certain loci of the metaxylem ring. Phloem elements are seen along the outer surface of the metaxylem elements, central pith cavity is surrounded by 3 layers of parenchymatous cells (Fig. 2-1.2).

Leaf

Macroscopical Characters

The leaf is fairly thick, radical leaves rosulate, narrow 0.5-1 cm long, cauline leaves 5-7 in a whorl at nodes, narrowly linear or acicular, 1-1.5 cm length, 0.2 cm width, smell pleasing and no specific taste.

Microscopical Characters

Transverse section of the leaf shows uneven surface and less prominent midrib. The upper and lower epidermal cells are 50 µm thick, some of the epidermal cells are mucilaginous and the mucilage oozes out as clouds from the epidermis. The mesophyll consists of 3 to 4 layers of wide, thin walled compactly arranged parenchyma cells (Fig. 3.1.2).

The midrib is not prominent, it is slightly bulged on the lower side and flat on the upper side. The vascular bundle of the midrib consists of two small discrete xylem strands and few phloem elements, vascular bundle is surrounded by a layer of bundle sheath cells, and outer to the hyaline sheath cells a layer of radially oblong cells containing chloroplasts are present.

Whole plant-Macerate: Whole plant maceration shows the following elements.

Fibres (Fig. 4.1):

Mostly narrow, long and cylindrical with thick walls and narrow lumen. Pits not evident up to 950 µm long and 15 µm wide.

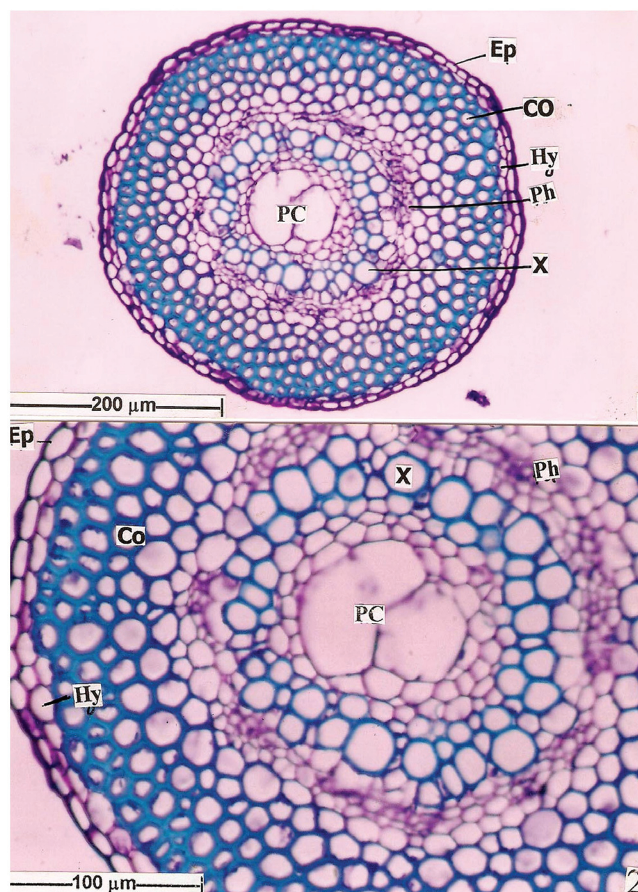

Figure 2: Anatomy of the stem
2.1 T.S. of stem -- entire views
2.2 T.S. of stem -- a sector enlarged
Pharmacognosy of a local market sample of Parpataka *Mollugo cerviana* (L.) Ser.
Legends – Fig. 1 and 2
Co – Cortex; GR – Growth ring; Px – Primary xylem; Sph – Secondary Phloem ; Sx – Secondary xylem; V – Vessel; XF – Xylem fibres; Ep – Epidermis ; Hy – Hypodermis; PC – Pith cavity; Ph – Phloem ; X – Xylem

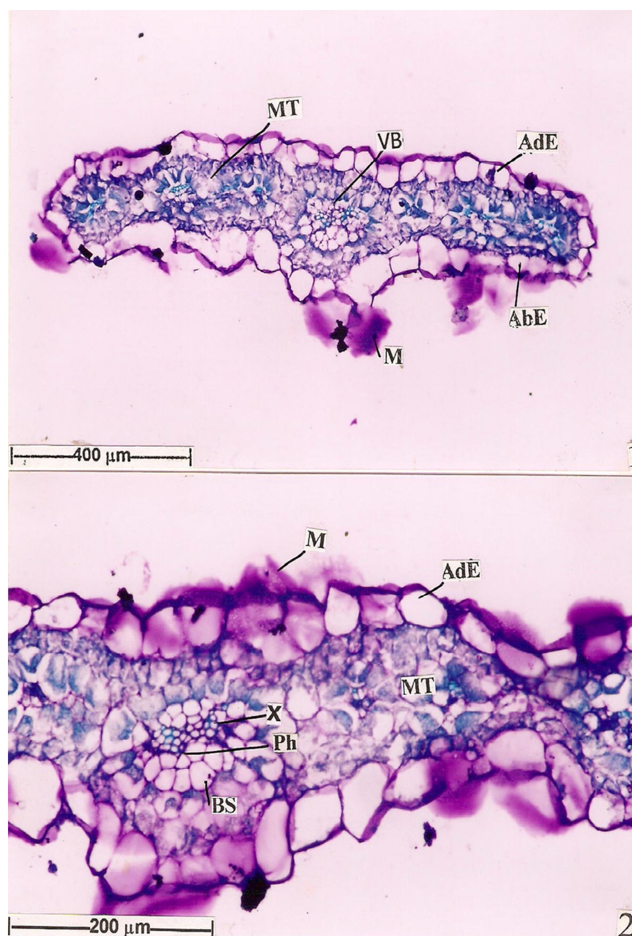

Figure 3: Anatomy of the leaf
3.1 T.S. of leaf entire view
3.2 T.S. of leaf midrib enlarged

Vessel elements (Fig. 4.2):

Long, narrow, cylindrical, perforation plate simple and oblique. Lateral wall pits circular, alternate. Vessel elements tailless or shortly tailed up to 250 µm long and 30 µm wide.

Epidermal cells (Fig. 5.1):

Cells large, amoeboid in shape, walls wavy thick and smooth.

Seed (Fig. 5.2):

Semicircular seeds with smooth seadcoat, embryo curved and horse – shoe shaped. Seed size 450 × 500 µm wide.

Seed Coat (Fig. 5.3):

Cells of the seed coat elongated, polyhedral, thick walled, cells arranged in longitudinal parallel rows. Outer cell layer consists of vertically oblong palisade cells.

Powder microscopy

In the powdered preparation, leaf epidermal peeling and seeds were observed. The adaxial epidermis in surface view has highly lobed cells, their anticlinal walls are thick and much undulate. Entire seeds were also visible in the powder; the seeds are flat on one side and hemispherical on the other side. The seed is 450 µm in horizontal plane, the surface of the seed is smooth and even (Fig.6).

Histochemical Tests

The sections were treated with different reagents and the observations are provided in Table-1.

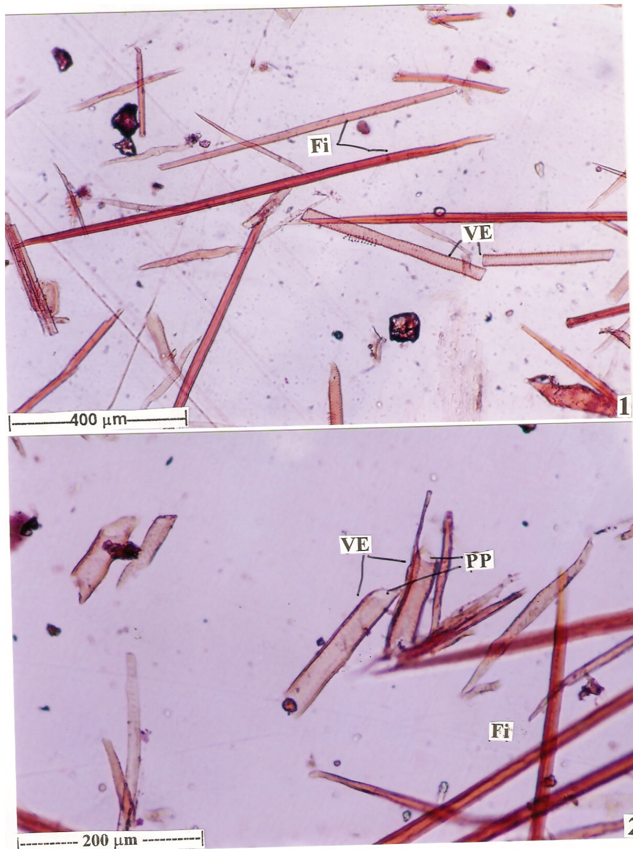

Figure 4:1–2: Whole plant macerate
VE – Vessel element; Fi – Fibres;
PP – Perforation plate
Legends – Fig. 3, 4
(AbE – Abaxial epidermis; BS – Bundle sheath; M – Mucilage; MT – Mesophyll tissue ; Ph – Phloem; VB – Vascular bundle; X – Xylem; AdE – Adaxial epidermal; S – Seeds).
Pharmacognosy of local market sample of Parpataka *Mollugo cerviana* (L.) Ser.

Powder Analysis

The powder is palegreen in color. It has been no characteristic odour and slightly bitter to taste. The results are given in table-2.

DISCUSSION

M. cerviana can be distinguished morphologically from *F. indica* in being small herbs, branchlets glacous, roots are slightly bitter to taste and microscopically widerzone of secondary xylem, growth ring and thick walled xylem fibres, stems are slender, filiform, slightly bitter to taste, presence of thick walled epidermal cells and cortex is made up of 3-4 layered scherenchymatous tissue. The central part is occupied by pith cavity. Leaves narrow or

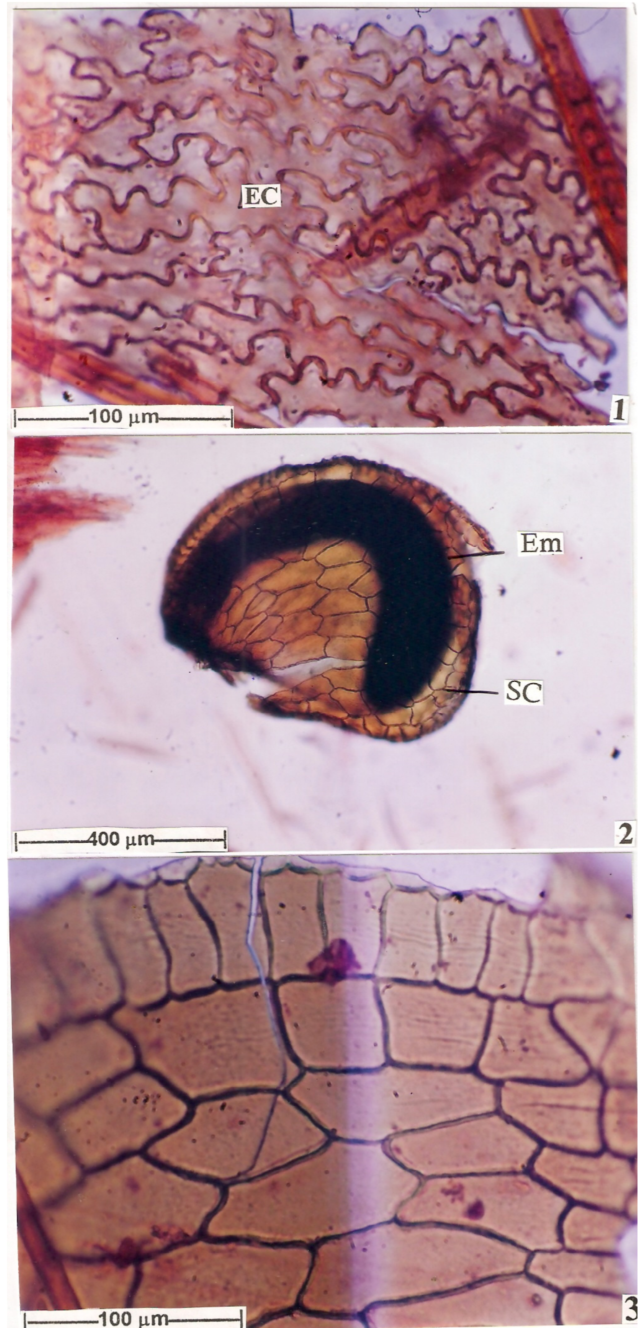

Figure 5. Mollugo cerviana
Whole plant macerate
1. Epidermal Cells in surface view
2. One seed with curved embryo
3. Seed coat cells enlarged
Legends – Fig. 5.
(EC – Epidermal cells; EM – Embryo; SC – Seed coat).
Pharmacognosy of a local market sample of Parpataka *Mollugo cerviana (L.)* Ser.

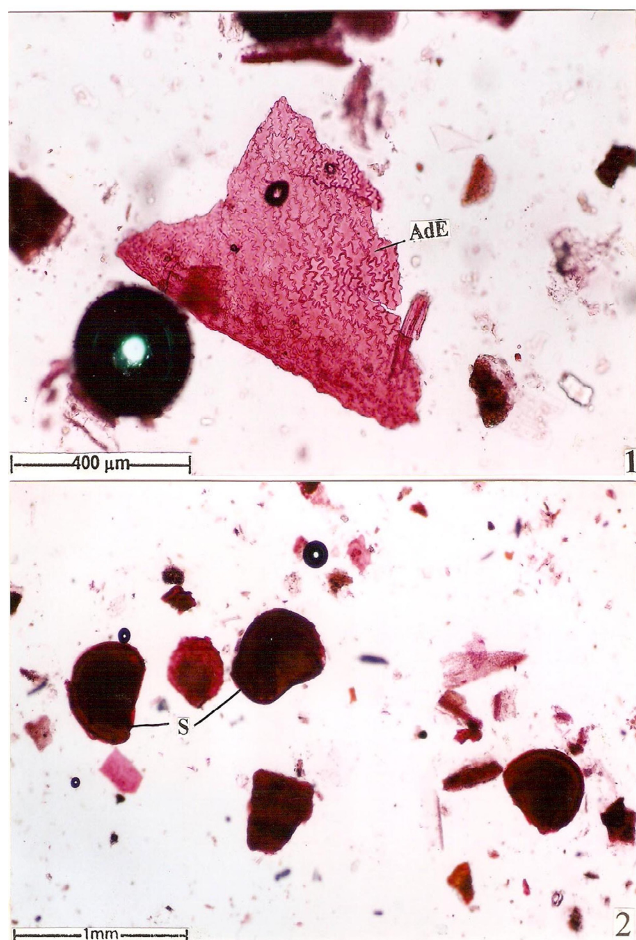

Figure 6: Powder microscopy
6.1 A fragment of epidermal layer
6.2 Seeds entire
Legends – Fig. 6
(AdE – Adaxial epidermis; S - Seeds).
Pharmacognosy of a local market sample of Parpataka *Mollugo cerviana* (L.) Ser.

linear and microscopically shows mucilaginous epidermal cells, mesophyll is made up of 3-4 layers of thin walled compactly arranged parenchymatous cells, vascular bundle is surrounded by a layer of radially oblong cells containing chloroplasts.

Table 2:

Treatment	Observation
Powder treated with water	Non-sticky
Powder shaken with water	Foam like froth
Powder treated with 5% aqueous NaOH	Dark olive
Powder treated with 60% aqueous sulphuric acid	Brown
Powder pressed between filter paper for 24 hours	No oil stain

A) Physical constants. The results are given Table-3.

Total ash (%)	9.775
Water soluble ash (%)	2.195
Alkalinity of water soluble ash (ml)	1.46
Acid insoluble ash (%)	1.415
Extract values(%)	
Alcohol soluble extract (% w/w)	2.141
Water soluble extract (% w/w)	4.10
Hexane soluble extract (% w/w)	0.66
Chloroform soluble extract (% w/w)	0.28

CONCLUSION

The South Indian Ayurvedic drug market sample of the Parpataka is evaluated for its identification, botanical, macro-, microscopic details of root, stem, leaf, whole plant macerete, powder microscopy, powder analysis, histochemical tests, physical constants and fluorescence studies of the drug identified as the *M. cerviana* (L.) Ser. it is not only used in Ayurvedic preparations but also used in the preparations of Siddha System of Medicine[14].

ACKNOWLEDGEMENTS

Our sincere thanks are due to the Regional Botanist, Botanical survey of India, Coimbatore for lending authentic herbarium specimens and the Director, PARC, Chennai for providing the necessary facilities to carry out this work.

Table-1: Histochemical Tests

Drug	Reagents	Test for	Reaction	Results
Section	Iodine solution	Starch	Blue colour	+
Section	Ferric chloride solution	Tannin	Black	+
Section	Sudan III solution	Oil globules	No effervescence	-
Section	Phloroglucinol + dil. HCl + Alcohol	Lignin	Magenta	+
Section	Conc. HCl	Crystals	No effervescence	-

+ = Present; - = Absent

B) Fluorescence Studies. The results are given in Table-4.

Experiments	Visible / Day light	UV Light	
		254 nm	365 nm
Drug powder	Pale green	Green	Brown
Drug powder + 1 N NaOH (aq.)	Brown	Green	Pale yellow
Drug powder + 1 N NaOH (alc.)	Brown	Yellowish green	Pale yellow
Drug powder + 1 N HCl	Brown	Pale green	Black
Drug powder + 50% H_2SO_4	Reddish brown	Black	Black
Drug powder + 50% HNO_3	Orange	Fluorescent green	Black
Drug powder + Picric acid	Dark olive (green)	Fluorescent green	Pale green
Drug powder + Acetic acid	Brown	Pale green	Black
Drug powder + Ferric chloride	Dark olive (green)	Pale green	Black
Drug powder + HNO_3 + NH_3	Reddish orange precipitate	Yellowish green	Green

REFERENCES

1. Sharma, P.V. *Dravyaguna Vijnana*, Varanasi (in Hindi), 1983.
2. Nesamony, S. *Oushadha Sasyanyal*, Trivandrum (in Malayalam), 1985.
3. Lakshmipathi, A. *One hundred useful drugs V. III*, Arogya Ashrama Samithi, Madras, p. 89, 1973.
4. Chunekar, K.C. *Plants of Bhava Prakasa*, National Board of Ayurveda, Ministry of Health and Family Welfare, New Delhi, 1999.
5. Bapalal Vaidya *Some controversial drugs in Indian Medicine*, Choukambha Orientalia Publishers, Varanasi, p. 207–211, 1985.
6. Anonymous, *The Wealth of India (Raw material)* Vol. **V-VII**. C.S.I.R. New Delhi, 1962.
7. Yoganarasimhan, S.N. medicinal plants of India, Tamil Nadu, **V-2**, p.262–472, 2000.
8. Ragacharyulu, D. *Floristic studies of Chittoor District*, Ph.D. Thesis, S.V. University, Tirupati. 1991.
9. Jain, S.K., and Rao R.R.. *Field and Herberiam methods*. Today and Tomorrow publishers, New Delhi, 1977.
10. Wallis, T.E. *Text book of pharmacognosy* (15th edition), T.A. Churchill, London, p. 571–582, 1985.
11. Johansen, D.A. *Plant micro-technique*. McGrow Hill, New York, p. 183–203, 1940.
12. Kokoski, C.J., Kokoski, R.J. and Salma, F.J., *Fluorescence of powdered vegetable drugs under ultra-violet radiation*. J. Am. Pharm. Asson., **47**: p. 715, 1958.
13. Chase, C.R. and Pratt R.J. Fluorescence of powdered vegetable drugs with particular resource to development of a system of identification. *J. Am. Pharm. Asso.*, **38**: p. 324–333, 1949.
14. Krebs, K.G., Heunsen, D. and Wimmer, H.W., IN (editor Stahl, E.), *Thin layer chromatography – A Laboratory handbook* (Second edition) London, p. 204–255, 855–909, 1969.
15. Harborne, J.B. *Phytochemical methods* 3rd edition. Chapman and Hall Publications, London, 1973.

Pharmacognostical Standardization of Stem Bark of *Adenanthera pavonina* L.

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Abstract

Adenanthera pavonina L. syn. Red Sandalwood, (Fabaceae) is an unarmed deciduous tree and and its bark is traditionally used for treatment of various disease conditions in gonorrhea, haematuria, ulcers, it is astringent, vulnerary and aphrodisiac in nature. An attempt has been made to highlight this folk herbal medicine through present study which will assist in standardization for quality, purity and sample identification. Various standardization parameters like morphological characters, microscopic evaluation, physico-chemical evaluations (foreign matter, loss on drying, ash values, extractive values), preliminary phytochemical screening, TLC finger print, qualitative HPTLC of the extract and fluorescence analysis of powdered crude drug were carried out and the qualitative parameters were reported. These studies provided referential information for correct identification and standardization of this plant material.

Keywords: *Adenanthera pavonina* L, standardization, phytochemical screening, pharmacognostical evaluation, HPTLC.

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INTRODUCTION

Medicinal plants are living and irreparable resource, which is exhaustible of over used and sustainable if used in the care and wisdom. The importance of medicinal plants has been over looked upon not only as a source of affordable health care but also as a source of income. According to WHO report, over 80% of the world population relies on the traditional medicine system largely plant, based for their primary health care needs.[1] In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of the traditional medicines. Indian Materia medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional system and folklore practice.[2-3] India has a rich heritage of traditional medicines and the traditional health care system have been flourishing for many centuries. It mainly consist of three major systems namely Ayruveda, Siddha and Unani system of Medicine.[4] In almost all the traditional system of medicine, the quality control aspect has been considered from its inspection itself by the Rishis and later by the Vaidya and Hakims. However, in modern concept it requires necessary changes in their approach. Quality control and quality assurance is an integral part of traditional medicines, which ensures that it delivers the required quantity of quality medicament.[5]

Adenanthera pavonina belongs to the family Leguminosae, subfamily Mimosoideae. The tree is known by a host of common names, including red-bead tree, red wood .The tree has been planted extensively throughout the tropics as an ornamental and has become naturalized in many countries. In India its origin is south, it has been cultivated in many parts in southern region.[6] In India, the plant is traditionally used for various medicinal purposes and its seeds are useful in vitiated conditions of vata and pitta. Besides this they are also used in curing gout, burning sensation, hyperdipsia, vomiting, fever and giddiness. Its heartwood is used as an astringent, aphrodisiac, haemostatic, and is useful in dysentery, and haemorrhages. Leaves are used to treat gout and rheumatism.[7, 8] The plant *Adenanthera pavonina* has been reported to contain a new five-membered lactone named pavonin with an exo-cyclic double bond has been isolated

from the methanol soluble part of *Adenanthera pavonina* [9], sterols (β-sitosterol, β-sitosterol-3β -D- glucoside), triterpenes (nonacosane & hentriacontane) [10] and saponins (sapogenins).[11] Earlier scientific investigation of *Adenanthera pavonina* showed that the crude extract has blood pressure lowering effect [12] antifungal, antioxidant and cytotoxic [13], and anti-inflammatory activities.[14] However, no scientific standards or pharmacognostical parameters are yet available to determine the quality of this crude drug. The present work therefore, attempts to report necessary pharmacognostical and standardization parameters of bark of .*Adenanthera pavonina.*

MATERIAL AND METHODS

Description of plant Adenanthera pavonina

The scientific name is derived from a combination of two Greek words *aden*,"a gland,"and *anthera*, "anther".It is found in Sub Himalayan tract, ascending upto an altitude of 1,200meters in Sikkim, West Bengal Assam, Meghalaya, Gujarat, Maharashtra, and is commonly known as Red wood. The main important constituents are flavonoid compounds (15). It is used as an antiseptic paste and also used to treat boils and inflammations (16). A medium- to large-sized deciduous tree, *Adenanthera pavonina* ranges in height from 6-15 m. It is generally erect, having dark brown to grayish bark, and a spreading crown. The seeds are hard-coated, lens-shaped, vivid scarlet in color, and adhere to the pods. The seed coat is smooth, shiny, bony, and very hard and generally has no fracture lines. . The pods are leathery, curve and twist upon dehiscence to reveal 8-12 showy seeds. The leaves are bipinnate. They are dark green in upper surface and blue green in lower surface. They become yellow with ageing. The bark is dark brown or grayish brown on outer surface and grayish white in inner surface. It is rough on old trees with longitudinal fissures. The small, yellowish flower grows in dense drooping rat-tail flower heads.They are small, creamy-yellow in color, and fragrant. Each flower is star-shaped with five petals. The wood is red in colour and extremely hard. It is durable and used for building purpose.

Collection and authentication

The fresh bark of the tree of *Adenanthera pavonina* L. was collected during the month of January 2009, from National Botanical Research institute, lucknow, India. For identification and Taxonomic authentication, sample of plant material was given to National Botanical Research Institute (NBRI) Lucknow, India. The text report from National botanical research institute, Lucknow, India and confirmed the authenticity of plant material sample was *Adenanthera pavonina* L. with voucher specimen no. NBRI -SOP-202 Receipt no. and date 19/72, 24-02-09. The fresh bark was used for the study of macroscopic and microscopical characters. Whereas collected plant materials were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values, and preliminary phytochemical investigation was studied as per standard methods.

Extraction of plant materials

100 gm coarse powdered of air dried bark of *Adenanthera pavonina* L. were packed in muslin cloth and subjected to soxhlet extractor for continuous hot extraction with distilled water, ethanol, petroleum ether and chloroform for 8 hrs separately. Then the each extracts were filtered and filtrate was evaporated to dryness. The percentage yield of the water, ethanol, petroleum ether and chloroform extracts was 4.18%, 2.72%, 1.68% and 1.15% respectively.

Macroscopic and microscopic studies

The macromorphology of the barks were studied according to standard methods. [17-19] Hand section of the bark was taken, stained and mounted following usual microtechniques [20] and representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).

Physicochemical analysis

Physicochemical analysis i.e. alcohol (90 % ethanol) and water soluble extractive values, fluorescent analysis [21, 22], total ash, acid-insoluble ash, water-soluble ash, swelling indices and foreign matter.[23] Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts and also loss on drying was noted.

Preliminary phytochemical screening

Preliminary phytochemical screening for the detection of various was carried out by using standard procedures described by Harborne [24] and Khandelwal [25]

Thin layer chromatography and high performance thin layer chromatography (HPTLC)

Thin layer chromatography studies of the ethanol and chloroform extracts carried out in various solvents at 30°C using Silica gel G as adsorbent and the $R_f$values were determined.[26] The same mobile phase was used for the HPTLC profiles of these extracts.

RESULTS

Macroscopic characters

The pieces of bark of *Adenanthera pavonina* L. are generally quills in shape or curved fragments, freshly collected bark is red in colour but on drying they turn from red to reddish brown in colour. The outer surface of the bark having more fissures. The inner surface is fine, smooth, and sparking, light red brown in color and fracture laminated (Figure 1). The bark is odorless and possesses slightly bitter astringent flavour.

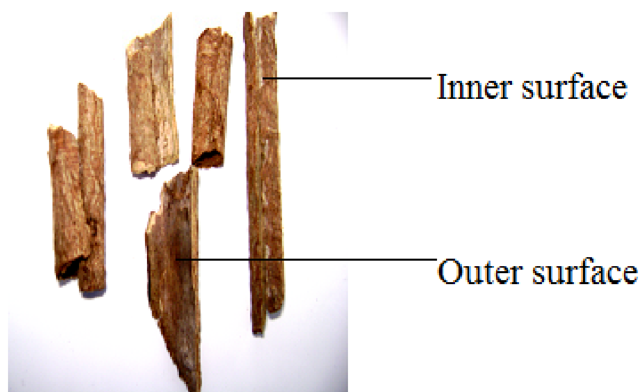


Figure 1 Morphological view of bark of *Adenanthera pavonina* L.

Microscopic characters

The transverse section of the bark shows the typical microscopical characteristics. Cork is the outer most covering; it consists of several layers of cork cells. They occur as regular rows of small slightly thick walled, flat polygonal cells, closely packed in radial rows that become dead at maturity, and generated from a cork cambium (Figure 2). In the periderm, the phellogen has continuity-type shape, phellem (cork) layer is oriented toward the outsides, while phelloderm layer is oriented toward the inside. Phellogen cells are rectangular and flattened, appearing radial in the cross section. The phellem is made of two or more layers of cells in a clear radial position but tangentially elongated due to the secondary growth. The phelloderm is of two or more layers of rectangular cells (Figure 2). The periderm and the cortex are separated by a layer of cells. The cortex region shows the presence of simple calcium oxalate crystals. Few mucilaginous cells are also present and they are solitary appear (Figure 3). The phloem is characterized by collapse cells structure .Scattered patches of the stone cells are found in the cortex region. The spherical parenchyma cells of the cortex region differentiate into medullary rays, which are tube-like towards the inner part of the cortex. The medullary rays tend to converge as they approach the inner stem as nearly paralleled lines (Figure 3).

Physicochemical parameters

The physico-chemical characters of powdered drug of bark of *Adenanthera pavonina* such as total alcohol soluble extractive, water soluble extractive, ash value, acid insoluble ash, water-soluble ash, loss on drying, swelling index, and foreign matter are presented in Table 1. The

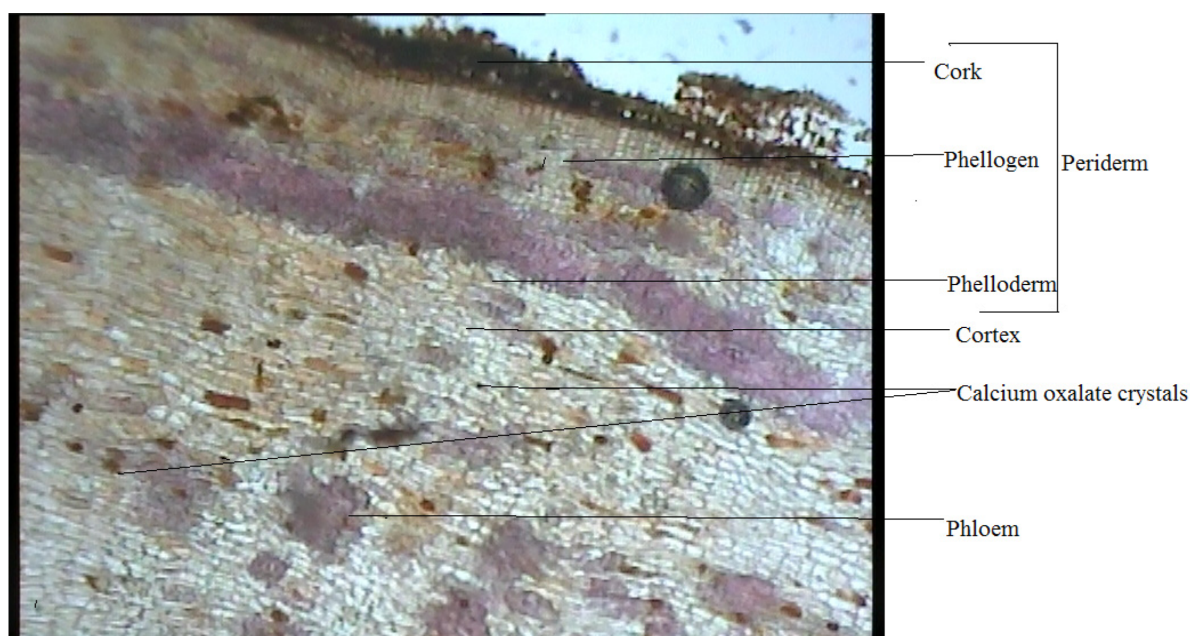


Figure 2 T.S of stem bark of *Adenanthera pavonina* L.

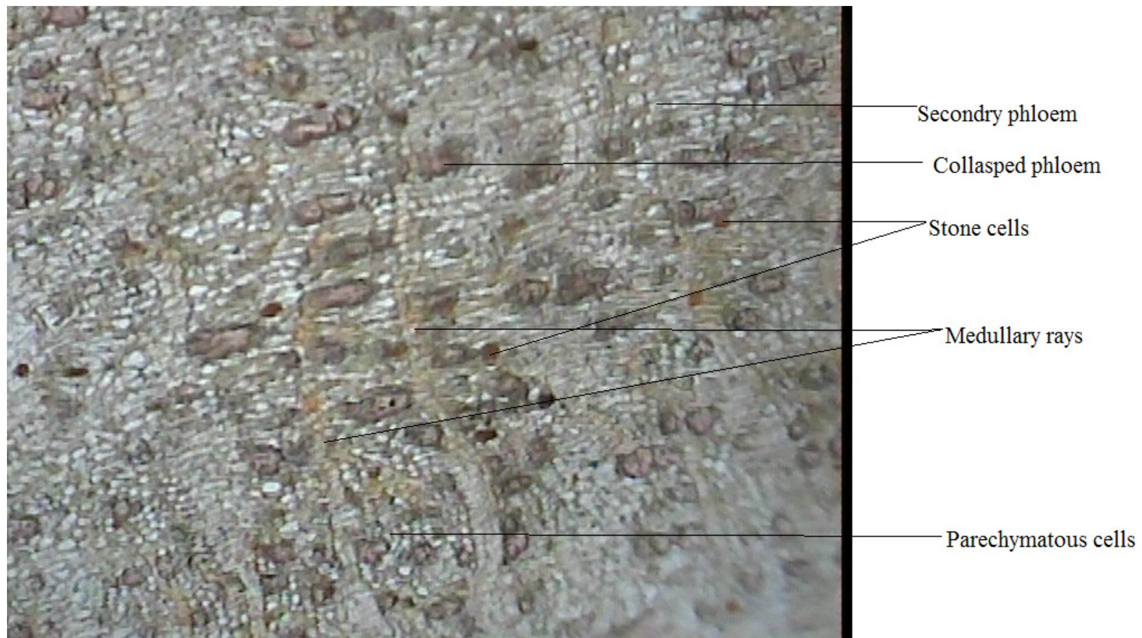
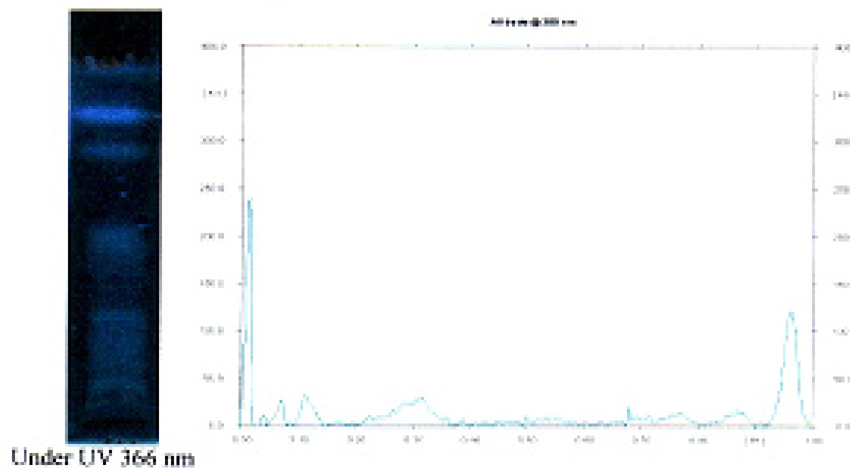


Figure 3 Cellular structure of T.S of stem bark of *Adenanthera pavonina* L.



Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area%	Assigned Substance
1	1	0.00 Rf	69.1 AU	0.01 Rf	242.5 AU	43.40%	0.03 Rf	0.0 AU	1836.9 AU	23.34%	unknown *
1	2	0.03 Rf	0.0 AU	0.04 Rf	11.4 AU	2.03%	0.05 Rf	2.2 AU	58.8 AU	0.75%	unknown *
1	3	0.05 Rf	6.3 AU	0.07 Rf	26.7 AU	4.78%	0.08 Rf	0.3 AU	307.6 AU	3.91%	unknown *
1	4	0.09 Rf	0.2 AU	0.11 Rf	31.4 AU	5.61%	0.14 Rf	0.4 AU	636.7 AU	8.09%	unknown *
1	5	0.21 Rf	2.4 AU	0.23 Rf	10.7 AU	1.91%	0.23 Rf	5.2 AU	108.7 AU	1.38%	unknown *
1	6	0.26 Rf	10.2 AU	0.29 Rf	24.3 AU	4.35%	0.30 Rf	22.9 AU	574.8 AU	7.30%	unknown *
1	7	0.30 Rf	23.7 AU	0.32 Rf	29.4 AU	5.25%	0.34 Rf	13.1 AU	631.9 AU	8.03%	unknown *
1	8	0.49 Rf	0.6 AU	0.50 Rf	10.8 AU	1.94%	0.51 Rf	2.1 AU	74.6 AU	0.95%	unknown *
1	9	0.67 Rf	2.8 AU	0.68 Rf	19.7 AU	3.53%	0.69 Rf	8.6 AU	128.4 AU	1.63%	unknown *
1	10	0.76 Rf	10.6 AU	0.77 Rf	14.1 AU	2.52%	0.80 Rf	1.4 AU	267.3 AU	3.40%	unknown *
1	11	0.84 Rf	3.2 AU	0.87 Rf	16.5 AU	2.96%	0.90 Rf	1.1 AU	482.1 AU	6.13%	unknown *
1	12	0.92 Rf	0.6 AU	0.96 Rf	121.3 AU	21.71%	0.99 Rf	0.9 AU	2762.5 AU	35.10%	unknown *

Figure 4 HPTLC Finger printing of ethanolic extract of bark of *Adenanthera pavonina* L. scanned at wavelength 366 nm.

fluorescence analysis of the powdered drug of *Adenanthera pavonina* in various solvents and chemical reagents was performed under normal and Ultra Violet (UV) light Table 2. The pH of 1 and 10% solution of powered drugs of *Adenanthera pavonina* was noted in Table 3.

Table 3. Determination of pH of the drug

Sample	pH
pH of 1 % solution	7.53
pH of 10 % solution	6.98

Preliminary phytochemical screening

The preliminary phytochemical investigation of the aqueous, ethanol, petrolieum ether and chloroform extracts of *Adenanthera pavonina* L. showed the presence of phytosterols, flavonoids, terpenoid saponins, carbohydrates, tannins, glycosides, alkaloids and proteins Table 4.

Table 1. Physicochemical parameters of stem bark of *Adenanthera pavonina* L.

Quantitative parameter	Values obtained (%) w/w
Alcohol soluble extractive	4.54
Water soluble extractive	12.60
Total ash	7.00
Acid insoluble ash	1.0
Water – soluble ash	6.5
Loss on drying	3.90
Swelling index	2.0
Foreign matter	0.273

Thin layer chromatography and high performance thin layer chromatography (HPTLC)

Thin layer chromatography of the aqueous and ethanolic extracts was carried out separately using chloroform: ethanol (7:3) for aqueous extract and chloroform: ethanol: ethyl acetate (7:2:1) for ethanolic extract as mobile phase respectively and the Rf values were recorded Table 5. The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots. TLC and HPTLC finger printing studies on ethanol extract showed presence of various phytoconstituents

Table 2. Fluorescence analysis of powdered stem bark of *Adenanthera pavonina* L.

Solvent used	Day light	UV Light (254 nm)	UV Light (366nm)
Benzene	brown	Light green	Light brown
Dist. water	Brown	Light green	Dark green
NaOH in water	Dark brown	Light green	Golden brown
NaOH in CH_3OH	Blackish brown	Dark green	Black
Chloroform	Golden brown	Yellowish green	Dark brown
Dil.HNO_3	Brown	Light brown	Golden brown
Acetone	Light brown	Light brown	Yellowish brown
Ethyl acetate	Light brown	Yellow	Yellowish green

Table 4. Qualitative analysis of phytochemicals in Adenanthera pavonina Linn.

Extracts	Pet ether	Chloroform	Ethanolic	Aqueous
Sterols	+	+	+	+
Tannins	-	-	+	-
Flavonoids	-	+	+	+
Proteins and amino acids	-	-	-	+
Glycosides	-	-	+	+
Phenols	-	-	+	+
Acidic compounds	-	-	-	-
Carbohydrates	-	-	+	+
Saponins	-	-	-	-
Alkaloids	-	+	+	+

+ Present - Absent

Table 5 Thin layer chromatography of Adenanthera pavonina Linn.

Test extract	Solvent system	Number of spots	R_f value
Aqueous extract	Chloroform:Ethanol (7:3)	2	0.75, 0.41
Ethanolic extract	Chloroform:Ethanol:ethyl acetate (7:2:1)	3	0.22, 0.47, 0.95

with their respective Rf values. The ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system Toluene: Ethyl acetate (8.5: 1.5) was ideal and gave well resolved sample peaks. The spots of the chromatogram were visualized at 366 nm (Figure 4).

DISCUSSION

As a part of standardization study, the macroscopical examination of bark of *Adenanthera pavonina* L. was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The macroscopical characters of the bark of plant can serve as diagnostic parameters. The extractive value, ash value, loss on drying and fluorescent analysis of the leaf extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The fluorescence analysis of the powdered drug from the bark of *Adenanthera pavonina* L. in various solvents was performed under normal and UV light. All the leaf extracts are examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent compounds. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may serve as a characteristic finger print for qualitative evaluation of bark.

CONCLUSION

Herbal drugs are derived from heterogeneous sources leading to variations. This makes the standardization of herbal medicines all the more important as erroneous results can cause variations in pharmacological and phytochemical studies. The pharmacognostic characters and phytochemical values reported in this paper could be used as a diagnostic tool for the standardization of this medicinal plant. Presence of adulterants can be easily identified using these parameters. The microscopic features could help in laying down micro morphological standards as per WHO guidelines for authentication of the drug. After present investigation it can be concluded that the standardization and preliminary phytochemical investigation study of *Adenanthera pavonina* L. bark yielded a set of standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy and toxicity of leaf, to establish as the drug.

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REFERENCES

1. Ambasta S.P. (Ed.), The useful plants of India. *Publications and Information Directorate*, (CSIR, New Delhi, India.1992).
2. WHO, *General guidelines for methodologies on research and evaluation of traditional medicine*, HO/EDM/TRM/2000.I, (Geneva, 2000) Pp. 74.
3. Agarwal S.S., and Paridhavi M., *Herbal Drug Technology*, (Universities Press private limited Hyderabad, 2007) Pp. 83, 625.
4. Kokate C.K., Purohit A.P., and Gokhle S.B., *Pharmacognosy*, (Nirali Prakashan Delhi, 2004) Pp. 1–2, 90, 99, 106, 597.
5. Mukherjee P.K., *Quality Control of Herbal Drugs*, 1st Edn, (Business Horizons Pharmaceutical Publishers, New Delhi, 2002) Pp 131–219.
6. *The wealth of India, National Institute of Sc.Communication and information resources*, vol-**1**, A-B,1948, pg.31
7. Khan Ali Irfan, Khanum Atiya, Herbal medicine for human diseases, (Ukaaz publications, Hyderabad, 2007) Vol.**3**, Pp 30.
8. Warrier P.K., *"Indian Medicinal Plants a compendium of 500 species"* edition-4th, (Orient Longman pvt.ltd 2003) Vol-**1**, Pp -58.
9. Ali M.S., Ahmed F., Azhar I., Pervez M.K., Pavonin: a new five-membered lactone from *Adenanthera pavonina* Linn. (Mimoaceae). *Nat Prod Res*. **19**(1): 37–40. (2005).
10. Ahmad Mesbah., Rehman U., Ataur M., Tabassum., Rumana., Naher Kamrun., Chemical constituents of leaves of *Adenanthera pavonina Journal of the Bangladesh Chemical society*,**15**(2): 194–199 (2002).

11. Yadav Neerja., Misra Gopal., Nigam S.K., Triterpenoids of *Adenanthera pavonina* bark. *Planta Med*. **29**: 176–179 (1976).
12. Aduragbenro D., Adedapo A., Yeside O., Osude., Adeolu A., Adedapo J., Olanrewaju Moody., Ayotunde S., Adeagbo., Olumayokun A., Olajide and Janet M.,Makind. Blood Pressure Lowering Effect of *Adenanthera pavonina* Seed extract on normotensive rats, *Rec. Nat. Prod.* **32**, 82–89 (2009).
13. Rodrigo S.K., Jayasinghe U.L.B., and. Bandara B.M.R., Antifungal, Antioxidant and cytotoxic activity of *Acronychia pedunculata* and *Adenanthera pavonina*. *Proceedings of the Peradeniya University Research Sessions*, Sri Lanka, Vol.12, Part I, 94–95 (2007).
14. Olajide O.A., Echianu C.A., Adedapo A.D., Makinde J.M., *Anti-inflammatory studies on Adenanthera pavonina seed extract*. **12**(2): 196–202 (2004).
15. Rastogi., and Mehrotra[a]., *Compendium Indian Medicinal plants*. Vol. **2**, PID, New Dehli, 1991) Pp. 23.
16. Chopra R.N, Nayar S.L, Chopra I.C., *Glossary of Indian medicinal plants*, (CSIR, New Dehli1956) Pp.6.
17. Brain K.R., and Turner T.D., *The Practical evaluation of phytopharmaceuticals*, (Wright-Scientechnica, Bristol,1975) Pp 7–9.
18. Mukherjee P.K., *Quality Control of Herbal Drugs*, (Business Horizon's Pharmaceutical Publishers, New Delhi, 2002) 138–141.
19. Evans W.C., *Trease and Evans Pharmacognosy*, 15th edition, (W.B. Saunders, Edinburgh London New York Philadelphia St Louis Sydney Toronto, 2002) 519–520, 545–547.
20. Johansen D.A., *Plant Microtechniques*. 1st edition, (McGraw-Hill Book Company, New York and London, 1940) 182–203.
21. Harborne J.B., Methods of extraction and isolation. *Phytochemical Methods*. (Chapman & Hall, London, 1998) Pp. 60–66.
22. Evans W.C., *Trease and Evans, Pharmacognosy*, 15th edition, (W.B. Saunders, Edinburgh London New York Philadelphia St Louis Sydney Toronto, 2002) Pp. 519–547.
23. Chase C.R., and Pratt R.J., Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. Am. Pharmacol. Assoc*, 1949, **38**, Pp. 32–33.
24. Harborne J.B., *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. 3rd edition, Springer (INDIA) Pvt. Ltd., New Delhi, 1998) Pp. 5–12.
25. Khandelwal KR, *Practical Pharmacognosy*, Technique and experiments, Nirali prakashan Delhi, Nineteenth Edi, appendix 1, 2008, pp. 183,
26. Stahl E., *Thin layer Chromatography-A Laboratory handbook*, 2nd edition (Springer India private limited, 2005) Pp. 53–83.