Phytochemistry and Pharmacological Properties of *Thunbergia laurifolia*: A Review

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ABSTRACT

Commonly known as blue trumpet vine or laurel clock vine, *Thunbergia laurifolia* is a popular ornamental vine in the tropics. Flowers are attractive with pale purplish-blue petals and a yellow throat. Leaves are heart-shaped with a pointed tip and slightly serrated leaf margin. In Thailand, leaves of *T. laurifolia* are believed to have detoxifying effects. They are used as an antidote for poisons and drugs, including the treatment of drug addiction. The plant has also been reported to have antioxidant, anti-diabetic, anti-inflammatory, and antipyretic properties. Local herbal companies are producing herbal teas and capsules of *T. laurifolia*, known as Rang Jeud in Thai. Compounds isolated from the leaves included iridoid glucosides, grandifloric acid, glucopyranosides, and derivatives of apigenin. Other compounds found in leaves and flowers were delphinidin derivatives, and phenolic acids of chlorogenic, caffeic, gallic, and protocatechuic. Current knowledge on the pharmacological properties of the species is reviewed. Properties reviewed include antioxidant, antimicrobial, antiproliferative, hepatoprotective, and anti-inflammatory activities, as well as detoxifying, anti-diabetic, and non-toxic effects.

Key words: Antioxidant, detoxifying, hepatoprotective, anti-diabetic, anti-inflammatory, non-toxic

INTRODUCTION

Thunbergia laurifolia Lindl. (Thunbergiaceae), or commonly known as blue trumpet vine or laurel clock vine, is native to India.[1,2] The species is grown as an ornamental plant and being a fast-growing vine, it has become an exotic weed in some countries. Its leaves are dark green, opposite, heart-shaped, with a pointed tip and slightly serrated leaf margin.[3-5] The leaf blade can grow up to 20 cm in length and 16 cm in width with a petiole up to 6 cm in length. Leaves are thin and bright green in colour when young, and tend to be darker green, thicker and slightly variegated as they mature (Figure 1). Borne on pendulous inflorescences, flowers are attractive, trumpet-shaped, with 5-7 rounded and pale purplish-blue petals, and a yellow throat (Figure 2). The flower is up to 8 cm long and 6-8 cm across. The plant produces round green stems and a tuberous root system. Propagation is from stem or root cuttings.

The plant flowers continuously throughout the year with flowers opening early in the morning and aborting in the evening of the same day.[3,4] Flowers are not scented. Carpenter bees are frequent visitors, creeping into the flowers for the pollen and nectar while black ants are present probably as nectar scavengers.

In Thai traditional medicine, leaves of *T. laurifolia* are used as an antidote for poisons and drugs including the treatment of drug addiction.[6,7] The plant has also been reported to have anti-inflammatory, anti-diabetic, and antipyretic properties.[8-10] Local herbal companies are producing and marketing herbal teas and capsules of *T. laurifolia*, known as Rang Jeud in Thai.

PHYTOCHEMISTRY

The phytochemistry of *T. laurifolia* leaves has been studied.[8] Two novel iridoid glucosides of 8-*epi*-grandifloric acid and 3'-*O*-β-glucopyranosyl-stilbericoside have been isolated, along with seven known compounds of grandifloric acid, benzyl β-glucopyranoside, benzyl β-(2'-*O*-β-glucopyranosyl)-glucopyranoside, 6-*C*-glucopyranosyl apigenin, 6,8-di-*C*-glucopyranosyl apigenin, (*E*)-2-hexenyl-β-glucopyranoside, and hexanol-β-glucopyranoside.

Leaves and flowers of *T. laurifolia* have been found to contain other bioactive phenolic constituents including delphinidin-3,

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DOI: 10.5530/pj.2011.24.1

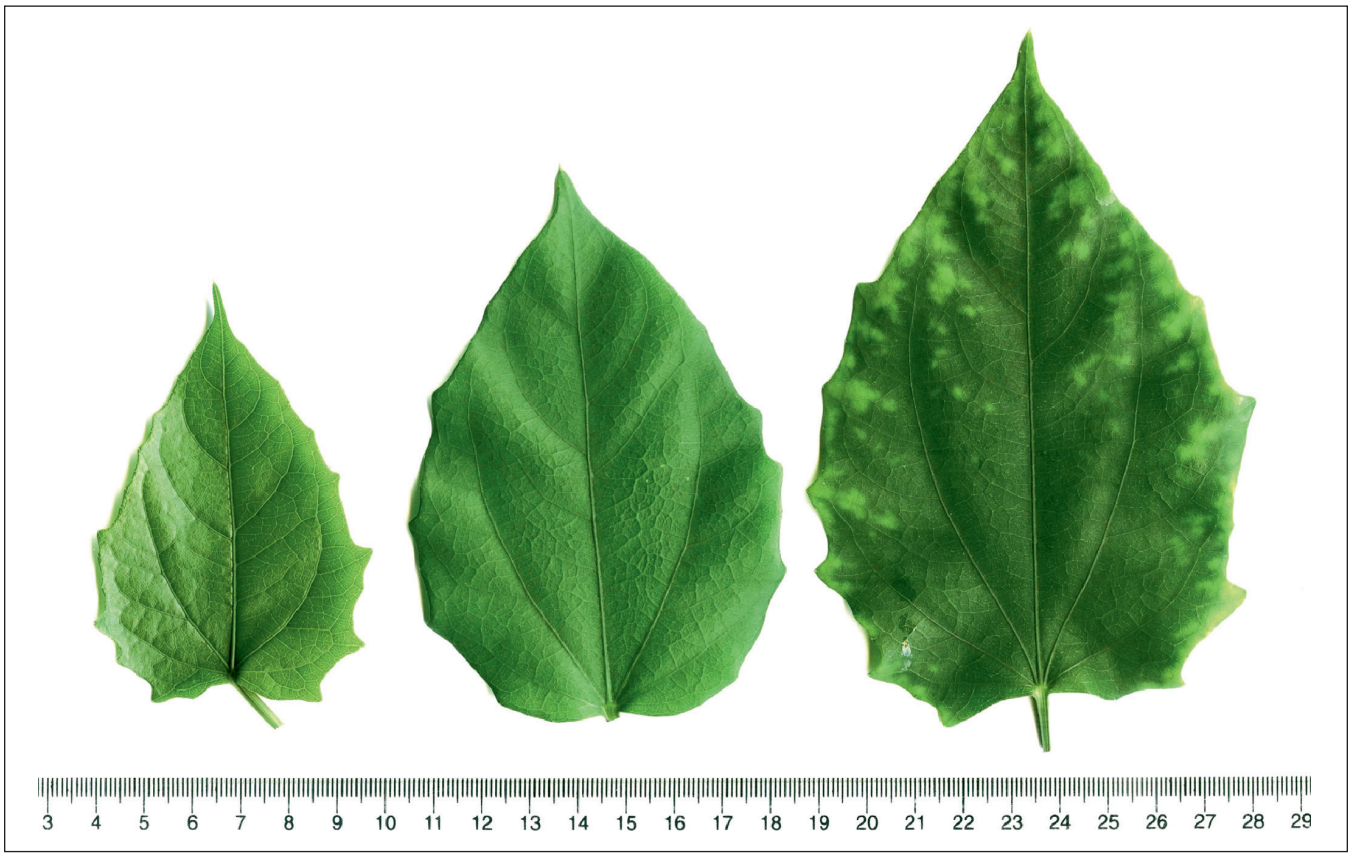

Figure 1: Young (left), developing (middle), and mature (right) leaves of *Thunbergia laurifolia*

Figure 2: Flowers of *Thunbergia laurifolia*

5-di-*O*-β-D-glucopyranoside, apigenin, apigenin-7-*O*-β-D-glucopyranoside, and chlorogenic acid.[6,12] A phenolic profiling of water extract of leaves of *T. laurifolia* showed the presence of apigenin and apigenin glucosides, as well as phenolic acids of caffeic, gallic, and protocatechuic.[12]

Proximate analysis of the contents of fibre, ash, protein, fat, and carbohydrate in leaves of *T. laurifolia* (dry weight) were 16.8, 18.8, 16.7, 1.68, and 46.0%, respectively.[13]

PHARMACOLOGY

Antioxidant activity

Water, ethanol, and petroleum ether extracts of dried leaf powder of *T. laurifolia* were evaluated for total phenolic content (TPC), free radical scavenging, and ferric reducing power (FRP).[14] Based on TPC, it was found that water extraction (2430 mg GAE/100 g) was the most efficient compared to ethanol (565 mg GAE/100 g) and acetone (142 mg GAE/100 g) extraction. The water extract also yielded the highest free radical scavenging with EC_{50} value of 0.13 mg GAE/ml, whereas ethanol and acetone extracts had EC_{50} values of 0.26 and 0.61 mg GAE/ml, respectively.

The water extract also showed the highest FRP (0.93 mmol/g), compared to extracts of ethanol (0.18 mmol/g) and acetone (0.04 mmol/g).

Screening of ethanol extracts from 134 species of edible Thai plants for superoxide inhibitive activity, leaf extract *T. laurifolia* was found to have moderate inhibition rates of 50-69% for total activity and xanthine oxidase inhibition.[15]

A study on the optimum time and efficiency of methanol extraction for leaves of *T. laurifolia* demonstrated that 1 h was the optimum extraction time.[3] TPC values for 0.5, 1.0, and 2.0 h were 418 ± 57, 721 ± 105, and 636 ± 71 mg GAE/100 g, respectively. Based on TPC, the first extraction extracted about 88% of the phenolic compounds. Yields of the second and third extractions were only 10.5 and 3.5%, respectively, suggesting that methanol is efficient in extracting leaves of *T. laurifolia*.

Variations in TPC between *T. laurifolia* leaves of different ages, collection times, and locations were also reported.[3] Developing leaves had the highest TPC of 513 ± 8 mg GAE/100 g, followed by young and mature leaves with values of 407 ± 11 and 298 ± 9 mg GAE/100 g, respectively. TPC values varied from 532 ± 8 to 795 ± 16 mg GAE/100 g for four batches of leaves collected from the same source in April and May 2004. Leaves collected from plants located in three different locations on the same day had significantly different TPC values of 543 ± 15, 734 ± 13, and 892 ± 8 mg GAE/100 g, suggesting variation between plants. Within plants, leaves and flowers had comparable phenolic content and free radical scavenging ability.

The effects of different drying methods on the antioxidant properties of *T. laurifolia* leaves have been reported.[3,4] Antioxidant properties investigated were TPC and ascorbic acid equivalent antioxidant capacity (AEAC). Leaves (2 g each) were each subjected to three different drying methods. Oven drying involved drying for 5.5 hours in an oven set at 50°C. Sun and microwave drying was for 16 h and 4 min, respectively. For oven and sun drying, TPC and AEAC declined 73 and 76%, and 80 and 89%, respectively. For microwave drying, TPC and AEAC gained 38-41 and 50-51%, respectively. Microwave-dried leaves remained green with a faint fragrance and when ground, the aromatic green-coloured tea produced a mild tasting green infusion. For the microwave-dried tea, hot water extraction yielded TPC and AEAC values that were 1.7-1.9 and 2.0-2.1 times higher than those of methanol extraction. When compared to other commercial teas, TPC, AEAC, and FRP values of the microwave-dried tea were 6.4, 8.7, and 9.3 times those of the commercial *T. laurifolia* tea, and were superior to teas of *Orthosiphon aristatus* and *Aspalathus linearis*.

Antioxidant properties of 13 commercial tropical herbal teas based on screening of their TPC, AEAC, and FRP have been reported.[16] Herbal tea of *T. laurifolia* was among the low antioxidant category together with herbal teas of *Alpinia zerumbet*, *Garcinia atroviridis*, and *Cymbopogon citratus*. Values of herbal tea of *T. laurifolia* (Rang Jeud) were 805 ± 50 mg GAE/100 g, 591 ± 29 mg AA/100 g, and 43 ± 5 mg GAE/100 g, respectively.

A study on the effects of various thermal and non-thermal drying methods on the antioxidant properties of leaves and teas of *T. laurifolia* showed remarkable differences.[17] Leaves of *T. laurifolia* (15 g) were shredded, and microwave-dried (1.5 min), oven-dried (3 h), freeze-dried (overnight), and freeze-withered (2 h). Dried leaves were extracted by steeping in hot water (1 h) to obtain the tea infusions.

Freeze withering and oven drying led to declines in TPC (85 ± 0.6 and 36 ± 2.3%) and AEAC (96 ± 2.4 and 25 ± 2.8%), respectively, compared to fresh leaves (Table 1). Values of freeze-dried leaves remained unchanged i.e. comparable to those of fresh leaves. Interestingly, values of microwave-dried leaves were 38 ± 3.2 and 84 ± 6.1% higher than those of fresh leaves, respectively.

Antioxidant properties of all *T. laurifolia* teas produced were significantly higher than those of the commercial Rang Jeud tea, with the exception of freeze-withered tea which had comparable properties.[17] Freeze-dried, microwave-dried, and oven-dried teas had TPC values

Table 1: Percentage water loss and gain/loss in total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of dried leaves in comparison with fresh leaves (fresh weight)

Drying method	Water loss (%)	Gain/loss (%)	
		TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Freeze withering	79 ± 0.9	−85 ± 0.6	−96 ± 2.4
Oven drying	78 ± 1.2	−36 ± 2.3	−25 ± 2.8
Freeze drying	80 ± 1.1	+0.4	−0.7
Microwave drying	79 ± 0.6	+38 ± 3.2	+84 ± 6.1

TPC and AEAC are means ± SD (n = 3). Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid.

Table 2: Total phenolic content (TPC) and ascorbic acid equivalent antioxidant capacity (AEAC) of tea infusions of *Thunbergia laurifolia* in comparison with the commercial tea (dry weight)

Tea infusion	TPC (mg GAE/100 g)	AEAC (mg AA/100 g)
Freeze-dried	3850 ± 127 [a]	4520 ± 100 [a]
Microwave-dried	3080 ± 202 [b]	3450 ± 273 [b]
Oven-dried	1800 ± 57 [c]	1590 ± 55 [c]
Commercial tea	577 ± 39 [d]	398 ± 22 [d]
Freeze-withered	488 ± 44 [e]	219 ± 63 [e]

TPC and AEAC are means ± SD (n = 3). Abbreviations: GAE = gallic acid equivalent and AA = ascorbic acid.

that were 6.7, 5.3, and 3.1 times, and AEAC values that were 11.4, 8.7, and 4.0 times that of the commercial tea (Table 2). Ranking of the teas based on antioxidant properties was of the order: freeze-dried > microwave-dried > oven-dried > commercial ~ freeze-withered. The various colours of *T. laurifolia* tea infusions produced from different drying methods are shown in Figure 3. TPC and AEAC values of 577 ± 39 mg GAE/100 g and 398 ± 22 mg AA/100 g of the commercial *T. laurifolia* (Rang Jeud) tea were significantly lower than values of 805 ± 50 mg GAE/100 g and 591 ± 29 mg AA/100 g reported earlier.[16] There is significantly difference in antioxidant values between different batches of the commercial *T. laurifolia* tea, suggesting that manufacturing procedures have not been standardised.

Antimicrobial activity

A total of 41 types of Thai medicinal teas were analysed for UV light-activated antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, and *Aspergillus fumigatus*.[18] Results showed that the ethanolic leaf extract of *T. laurifolia* did not have any antibacterial or antifungal activities with the exception of UV light-activated activity against *B. subtilis*.

Antiproliferative activity

Ethanolic extracts of nine Thai medicinal plants were screened for antiproliferative activity against SKBR3 human breast adenocarcinoma cells using the methylthiazoletetrazolium (MTT) assay.[19] Leaf extract of *T. laurifolia* did not show positive antiproliferative activity. Similarly, dried leaf powder extract of *T. laurifolia* showed very weak or no cytotoxic activity against BHK and L929 normal cells, and HepG2 and Caco-2 cancer cells using the MTT assay.[14]

Hepatoprotective activity

Leaf extract of *T. laurifolia* protected mice from hepatic injury induced by ethanol.[20] The hepatoprotective activity of aqueous extracts *T. laurifolia* against ethanol induced liver injury in rats and in primary cultures of rat hepatocytes has also been reported.[21] The extract at appropriate doses increased cell viability of primary cultures of ethanol-treated rat hepatocytes by 2-3 folds and decreased release of alanine transaminase (ALT) and aspartate transaminase (AST). It also promoted rat liver recovery after 14 days of ethanol treatment as reflected by the decrease in severity of rat liver injury and the normalization in hepatic triglyceride, ALT, and AST levels.

Detoxifying effects

The effects of *T. laurifolia* on endogenous dopamine release from rat striatal slices in comparison with those of amphetamine have been investigated.[6] The effect of hot water extracts of dried *T. laurifolia* leaves on K^+ stimulated dopamine release from rat striatal slices were compared with amphetamine using HPLC with electrochemical detection. Results showed that *T. laurifolia* may stimulate dopamine release in a similar manner to amphetamine.

A follow-up study was conducted to determine whether *T. laurifolia*, which has been used in the treatment of toxicity and addiction, can alter rat brain region activity using *in vivo* functional nuclear magnetic resonance imaging.[7] It was reported that the methanolic leaf extract of *T. laurifolia*

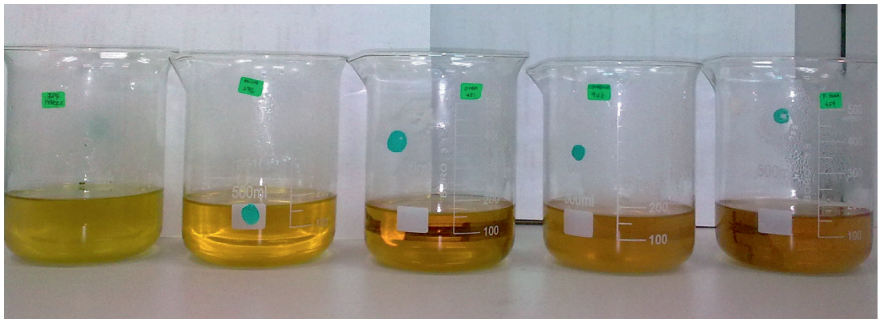

Figure 3: Infusions of *Thunbergia laurifolia* herbal teas

increased signal intensity in various parts of the brain, similar to the effects of cocaine or amphetamine. There was a slight decrease in arterial blood pressure. Results showed that *T. laurifolia* can stimulate brain activity in a manner similar to addictive drugs such as amphetamine and cocaine. However, it remains to be confirmed whether *T. laurifolia* itself can cause addiction or not or whether the effects are indeed relevant to reports that the plant can treat drug addiction.

The effects of aqueous leaf extract of *T. laurifolia* in alleviating lead poisoning in the brain of mice have been studied.[22] Results showed that the extract can reduce neuronal cell death and memory loss caused by lead uptake in mice. The extract was found to restore the levels of caspase-3 activity, and maintain total antioxidant capacity and antioxidant enzymes in the brain.

The protection of *T. laurifolia* ethanolic leaf extract against lead toxicity in the Nile tilapia (*Oreochromis niloticus*) has been reported.[23,24] When supplemented in fish food, the extract had the ability to protect the fish against lead toxicity after 28 days of treatment. The fish had reduced levels of lead in the liver and muscle, and showed improved growth performance, blood chemistry, hematology, and histology.

The detoxifying effects of aqueous *T. laurifolia* leaf extract on cadmium-induced toxicity in rats have been investigated.[25] Two groups of six rats each were injected with cadmium chloride solution at 1.0 mg/kg body weight for 20 days. Injected rats fed with drinking water with 0.1 mg/ml of *T. laurifolia* leaf extract (group 2) had significantly higher body weight than those fed with only drinking water (group 1 as control). The rats in group 2 did not show histopathological changes in the kidney that were observed in the control group. The study demonstrated that *T. laurifolia* leaf extract can protect against cadmium-induced structural damage of rat kidney.

Another related study investigated the detoxifying effects of aqueous *T. laurifolia* leaf extract on paraquat-induced toxicity in rats.[26] It was reported that the rats treated with the plant extract had higher survival rates and lower levels of plasma malonaldehyde.

Anti-diabetic effects

The anti-diabetic effects of aqueous leaf extract of *T. laurifolia* have been studied.[10] Results showed that a 15-day treatment with the extract (60 mg/ml/day) decreased levels of blood glucose in diabetic rats. The recovery of some β-cells was found in diabetic rats. Whether *T. laurifolia* leaf contains insulin-like substance(s) which directly act as hypoglycemic agents, or contains substances that induce the regenerative process of β-cells remains to be investigated.

Non-toxic effects

A chronic toxicity study on *T. laurifolia* aqueous leaf extract on Wistar rats showed the extract at doses ranging from 20 to 2,000 mg/kg/day did not affect their body weight, food consumption, behavior, and general health.[9] The extracts did not produce cumulative toxic signs and fatal effects. It was suggested that effects of prolonged oral administration of the extract need to be monitored. In an earlier toxicity study of aqueous leaf extract of *T. laurifolia* in mice at 1, 2, 4, and 8 g/kg/day, it was reported that no mice died during the first month, suggesting that the extract is non-toxic, effective, and safe for consumption.[27]

Anti-inflammatory activity

The anti-inflammatory efficacy dose of the aqueous leaf extract of *T. laurifolia* (2.5 g/kg) has been reported to be two-fold that of *Garcinia mangostana* rind extract (5.5 g/kg).[27] Alcohol and hexane leaf extracts of *T. laurifolia* possess anti-inflammatory activity against carageenin-induced paw edema in mice.[28]

CONCLUSION

The growing scientific interest in *T. laurifolia*, a medicinal plant in Thailand, stems from traditional belief that the species has detoxifying effects, and can used as an antidote for poisons and drugs, including the treatment of drug addiction. Local herbal companies are producing and marketing herbal teas and capsules of *T. laurifolia*, known as Rang Jeud in Thai. In recent years, scientists in Thailand have conducted much research on the phytochemistry and pharmacological properties of the species. Properties included antioxidant, antimicrobial, antiproliferative, hepatoprotective, and anti-inflammatory activities, as well as detoxifying, anti-diabetic, and non-toxic effects. Some research interest has also been generated in Malaysia on the antioxidant properties of leaves and herbal teas of *T. laurifolia*. Recently, analyses of the infusion characteristics, sensory attributes, and consumer acceptability of *T. laurifolia* herbal teas produced by microwave drying, oven drying, freeze drying and freeze withering have been conducted, with comparisons to the commercial Rang Jeud tea from Thailand. To date, this report represents the first review of the phytochemistry and pharmacological properties of *T. laurifolia*.

ACKNOWLEDGEMENTS

This review forms part of the research project on the effects of different drying methods on the antioxidant properties of leaves and herbal teas of *T. laurifolia* conducted by three final year students of the Faculty of Applied Sciences, UCSI University. The project also included evaluations of the infusion characteristics, sensory

attributes, and consumer acceptability of *T. laurifolia* herbal teas. The support of the faculty and university is gratefully acknowledged.

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Kyllinga nemoralis (Hutch & Dalz) (Cyperaceae): Ethnobotany, Phytochemistry and Pharmacology

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ABSTRACT

Many herbal remedies have so far been employed for the treatment and management of various ailments since the beginning of human civilization. Kyllinga nemoralis (Hutch & Dalz) (Cyperaceae) is a plant widely used throughout the world and frequently used for its anti-venom property. The aim of this review was to collect all available scientific literature published and combine it into this review. The present review comprises the ethanobotanical, phytochemical and pharmacological potential of *Kyllinga nemoralis.* The present review includes 19 references compiled from major databases as Chemical Abstracts, Science Direct, SciFinder, PubMed, Dr. Dukes Phytochemical and Ethnobotany. An exhaustive survey of literature revealed that flavonoids, saponins, phenols, terpenes, lipids and glycosides constitute major classes of phytoconstituents of this plant. Pharmacological reports revealed that it is having analgesic, antidiabetic, anticancer, antioxidant, antimicrobial, hepatoprotective and antimalarial properties. *Kyllinga nemoralis* seems to hold great potential for in-depth investigation for various biological activities. Through this review, the authors hope to attract the attention of natural product researchers throughout the world to focus on the unexplored potential of *Kyllinga nemoralis*, and it may be useful in developing new formulations with more therapeutic value.

Key words: Ethnobotany, Phytochemistry, Pharmacology, Antivenom, *Kyllinga nemoralis*

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs. De Pasquale, 1984 A. De Pasquale, Pharmacognosy: the oldest modern science, *Journal of Ethnopharmacology* **11** (1984), pp. 1-16. Abstract | PDF (1361 K) | View Record in Scopus | Cited By in Scopus (15) In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems.[1] The Indian subcontinent The Indian sub-continent comprising of the countries India, Pakistan, and Bangladesh is the site of one of the oldest civilizations, and it has seen the development of many traditional health care systems. Their development was supported by the great biodiversity in flora and fauna due to variations in geography and climate.[2] Many weedy plants posses medicinal and therapeutic and therapeutic activities.[3, 4]

The Cyperaceae family comprising of monocotyledonous flowering plants known as sedges, which superficially resemble grasses or rushes. The family is large, with some 5,500 species described in about 109 genera. These species are widely distributed, with the centers of diversity for the group occurring in tropical Asia and tropical South America. Members of the family Cyperaceae are called Motha as a folkore name in different parts of the country and used as ethno medicinal plants for treatment of diverse ailments.[5] Some well-known sedges include the water chestnut (*Eleocharis dulcis*) and the papyrus sedge (*Cyperus papyrus*), from which the Ancient Egyptian writing material was made. This family also includes cotton-grass (*Eriophorum*), spike-rush (*Eleocharis*), sawgrass (*Cladium*), nutsedge or nutgrass (*Cyperus rotundus*, a common lawn weed), the large genus of *Carex*, and white star sedge (*Rhynchospora colorata*) and Whitehead spike sedge (*Kyllinga nemoralis).* This review aims at describing the traditional uses, phytochemical profiles

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DOI: 10.5530/pj.2011.24.2

and therapeutic potential of various parts of *Kyllinga nemoralis*, which has been used in traditional practice for many years.

CLASSIFICATION[6]

- Domain: Eukaryota
- Kingdom Plantae – Plants
- Subkingdom Tracheobionta – Vascular plants
- Superdivision Spermatophyta – Seed plants
- Division Magnoliophyta – Flowering plants
- Class Liliopsida – Monocotyledons
- Subclass Commelinidae
- Order Cyperales
- Family Cyperaceae – Sedge family
- Genus *Kyllinga* Rottb. – spike sedge
- Species *Kyllinga nemoralis* (J.R. Forst. & G. Forst.) Dandy ex Hutch. & Dalziel – whitehead spike sedge

DESCRIPTION OF *KYLLINGA NEMORALIS*

Kyllinga nemoralis (Hutch & Dalz) (Family; Cyperaceae) is a perennial herb, grass-like in habit, propagated by seed and a creeping rhizome with many synonyms and common names. Synonyms include *Cyperus kyllingia* Endl, *Kyllinga monocephala* Rottb and *Kyllinga cephalotes* (Jacq.) and Common names include Whitehead spike sedge, white kyllinga, white water sedge, white-flowered kyllinga, poverty grass. Grow chiefly in marshy and wet places and is well distributed over all parts of the world. This plant is commonly known as Apavisha, Nirbishi and Velutta nirbasi.

It is found in waste places, open grasslands, etc., at low and medium altitudes. It is pantropic in distribution. The plant is more or less glabrous, arising from creeping rootstocks. The stems are usually solitary, 10 to 40 centimeters high. The leaves are up to 15 centimeters in length or longer, 3 to 4 millimeters wide; with the bracts similar. The spikes are ovoid, simple, white, 8 to 13 millimeters long. The spikelets are very numerous, 3 to 3.5 millimeters long, the flowering glume distinctly winged along the keel. The fruit is an achene, approximately 1.2-1.5 mm long x 0.5-0.7 mm wide.[7]

ETHNOBOTANY

Kyllinga nemoralis leaves and rhizomes contain many biologically active chemicals, and extracts from those tissues have been used in traditional folk medicine to treat many diseases and conditions. The plant leaves are traditionally used for the relief of malarial chills, pruritus of the skin, and thirst due to fever and diabetes.[8] In India plant leaves are used as anti-venom.[9, 10] The rhizomes of the plant are fragrant, sweet, refrigerant, antidiarrhoeal, diuretic, stomachic, and expectorant.[11, 12] The paste of rhizomes mixed with milk is used internally for worm infection.[13] It is also used in fever, hepatopathy, splenopathy, diabetes and tumours.[14]

PHYTOCHEMISTRY

Only a few studies have reported on the Phytochemistry of *K. monocephala*. Underground parts contain essential oils rich in terpenes α-cyperone, β-selinene, and α-humulene.[15] The methanolic and aqueous extract from the plant leaves were positive for terpenoids, saponins and phenolic compounds.[16] More recently, ethanolic extract of the rhizomes possesses flavonoids, triterpenoids and glycosides and the petroleum ether extract was found to possess triterpenoids and glycosides.[17] Essential oil from fresh aerial parts by hydrodistillation from *Cyperus kyllingia* Endl. was analyzed by a GC, GC-MS. Twenty-three compounds were identified, mainly of oxygenated sesquiterpenes, particularly sesquiterpene hydrocarbons, and carboxylic acid. The most representative compounds were α-cadinol, caryophyllene oxide, α- muurolol, α-humulene, and α-atlantone.[18]

PHARMACOLOGICAL SCREENING

Analgesic activity

The analgesic activity of the methanol extract of the leaves of *Kyllinga monocephala* Rottb. (Cyperaceae) was evaluated using

the acetic acid-induced writhing test on mice and was found to significantly reduce the number of writhes in mice by half. Following a bioassay-guided fractionation scheme, statistically significant analgesic activity was observed with both the hexane and ethyl acetate partitions.[19] In another report the methanol extract of *K. monocephala* was found to significantly reduce the number of writhes in mice administered intraperitoneally with acetic acid to induce abdominal constriction.[16]

HEPATOPROTECTIVE ACTIVITY

Hepatoprotective activity of ethanolic and petroleum ether extracts of rhizomes of *Kyllinga nemoralis* was evaluated against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats at a dose 100 and 200 mg/kg, p.o. Both extracts showed significant hepatoprotection when compared to control, similar to standard silymarin. Histology of liver sections also revealed that the extracts protected liver from injury. Ethanolic extract possesses flavonoids, triterpenoids and glycosides and the petroleum ether extract was found to possess triterpenoids and lipids. The hepatoprotective effect produced may be probably due to the triterpenoids, which is common in both of these extracts.[17]

HYPOGLYCEMIC ACTIVITY

The hypoglycemic activity of Fresh plant infusion of *Kyllinga nemoralis* was monitored using the Oral Glucose Tolerance Test. Screening of the Blood glucose level of the animals was performed by the glucose oxidase method using a commercially available glucometer. *Kyllinga nemoralis* exhibited significant hypoglycemic activity when given 15 min after glucose load.[16]

ANTIMALARIAL, ANTICANCER AND ANTIMICROBIAL ACTIVITIES

Essential oil from fresh aerial parts by hydrodistillation from *Kyllinga nemoralis* was evaluated for antimalarial, anticancer and antimicrobial Activities. Antimalarial activity against *P. falcipalum* (K1) was determined by microculture radioisotope Techniques. The anticancer activity tested against the NCI-H187 cells. The preliminary antimicrobial activities were also evaluated using the agar diffusion method. The microorganisms used were: *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923, *Pseudomonas aeruginosa* ATCC27553, *Candida albican, Aspergillus flavus* and *Trichophyton mentagrophyte*. The oil showed significant activities against *P. falcipalum* (K1) and NCI-H187 (Small Cell Lung Cancer) with the IC50 values of 7.52 and 7.72 µg/mL, respectively. The potent activities of the oil might be attributable to its high sesquiterpene content.[18]

CONCLUSION

Kyllinga nemoralis is a wealth of indigenous knowledge and traditional uses have been documented for this species. While this review has attempted to unite the relevant information for this species the data clearly suggests future research priorities. Convincing ethnopharmacological evidence is presented alluding to the extensive use of *Kyllinga nemoralis* as antivenom. It is interesting to note that the earlier scientific investigations of this plant, *Kyllinga nemoralis*, showed the crude extracts exhibited analgesic, antimicrobial, hypoglycemic, anticancer, hepatoprotective and antimalarial properties. This review revealed that flavonoids, triterpenoids especially sesquiterpenes, glycosides, saponins, phenolic compounds and lipids constitute major classes of phytoconstituents of this plant. Monoterpenes, Polyphenols, saponins and flavonoids are well known for their biological properties and although a suite of compounds belonging to this class of phytochemicals have been identified, very few have been subjected to pharmacological assays. This plant can become important sources of novel drugs and lead compounds.

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Pharmacognostic Studies on the Leaves of *Dyschoriste Perottetii* Nees

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ABSTRACT

To ensure reproducible quality of herbal products, proper control of starting material is important. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. *Dyschoriste perrottetii* Nees (Family-Acanthaceae) is an important medicinal plant used in various ways in the treatment of microbial infections, fever, measles and pains. Macroscopic, microscopic and chemo-microscopic studies of powdered and anatomical sections of the leaf were carried out using standard methods. This is necessary, for the purpose of identification and monograph preparation. The result shows diacytic stomata on the lower and upper surface, surrounded by wavy walled epidermal cells, unicellular covering trichomes, calcium oxalate crystal, which are mostly single and prismatic, lignified fibres and a characteristic collenchyma cells below the epidermis. Chemo-microscopic examination revealed the presence of starch, tannin, mucilage and cellulose. Quantitative evaluation of the powdered leaves gave moisture content of 7.5 %, total ash 12.5 %, water soluble ash 5.3 %, acid insoluble ash of 4.0 %, and alcohol extractive and water soluble extractive of 31.2 and 21 .08 % respectively. These findings are of importance in the establishing diagnostic indices for the identification, Result could be used for identification and preparation of monograph on the plant.

Key words: *Dyschoriste perottetii*, macroscopy, microscopy, pharmacognostic evaluation.

INTRODUCTION

The plant *Dyschoriste perrottetii* Nees (Family-Acanthaceae) is a shrub of about half a meter high, with branches and square woody stem rooting at lower nodes.[1] It is widely distributed in the tropics frequently in temperate and completely absent in artistic region.[2] In Nigeria among the Hausas and Fulani communities, it is commonly known as *fidda hakukuwa* the plant is used in traditional medicine for easy labour and in treatment of yellow fever and measles and the seeds used for the removal of foreign material in the eyes.[3] Members of the Acanthaceae are of used for the relief of pain during child birth.[4] Pharmacological and biological study of the family shows that some members exert anticholinestrase activity, histamine antagonist, cardiac depressants, antimicrobial and antifungal effects.[5] Recently some were found to exhibit antitumour activity.[6] Preliminary phytochemical screening on the herb revealed the presence of phenolic compounds, alkaloids, steroids, saponins and tannin.[3] It was deemed of interest to investigate this plant pharmacognostically such as macroscopical, microscopical and other diagnostic character of the leaves of *Dyschoriste perrottetii* Nees, with a view of preparing monograph for its proper identification and inclusion in the pharmacopoeia.

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DOI: 10.5530/pj.2011.24.3

MATERIAL AND METHODS

Plant collection and identification

The plant was collected in February 2009 from Nsukka, Enugu State, Nigeria. It was identified and authenticated by Mr. A. Ozioko, a taxonomist of the Bio-resources and Development Conservation Programme Centre (BDCP) Nsukka and a Voucher Specimen (UN/PCOG/09/392) deposited in the Herbarium of Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka.

Macroscopical Examination

The macroscopical features of the leaves were studied using both the fresh and dried plant collected as described by Evans.[7]

Microscopical Examination

The powdered and transverse section of the leaf was employed for this study; to carry out quantitative and qualitative studies using the method employed.[7] Chemo-microscopical examination was carried out to detect the presence or absence of various chemical compounds such as starch, cellulose, tannins, and lignin, fat and oil, mucilage and calcium oxalate crystals.

Phytochemical studies

The preliminary phytochemical screening of the leaf powder was performed following standard qualitative chemical tests[7,8] in other to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance which include; alkaloids, tannins, flavonoids, saponins, glycosides, proteins, fats and oils, steroids and carbohydrates.

Quantitative microscopy

The moisture content of the powdered leaves was determined by loss on drying method.[8] The ash value, acid insoluble ash and water—soluble ash was determined as determined as described.[9] The water and alcohol extractive value were obtained using the method outline.[8]

RESULTS

Macroscopical examination

The leaves are simple, opposite. The shape is lanceolate with 2.5-5.0 cm wide and 6-12 cm long. The base of the leaf is decurente. The leaves are glabrous dark green in with apex sub acute, the margin shallowly wavy, reticulate venation, smooth and soft texture and petiole about 1.0-2.2 cm . It has characteristic, agreeable odour and slightly bitter.

Microscopical Examination

The microscopical features of the fresh and leaves powder were described as follows; diacytic stomata numerous on lower epidermis and moderate on upper epidermis, unicelluler covering trichome 4-12 μm in size, phloem

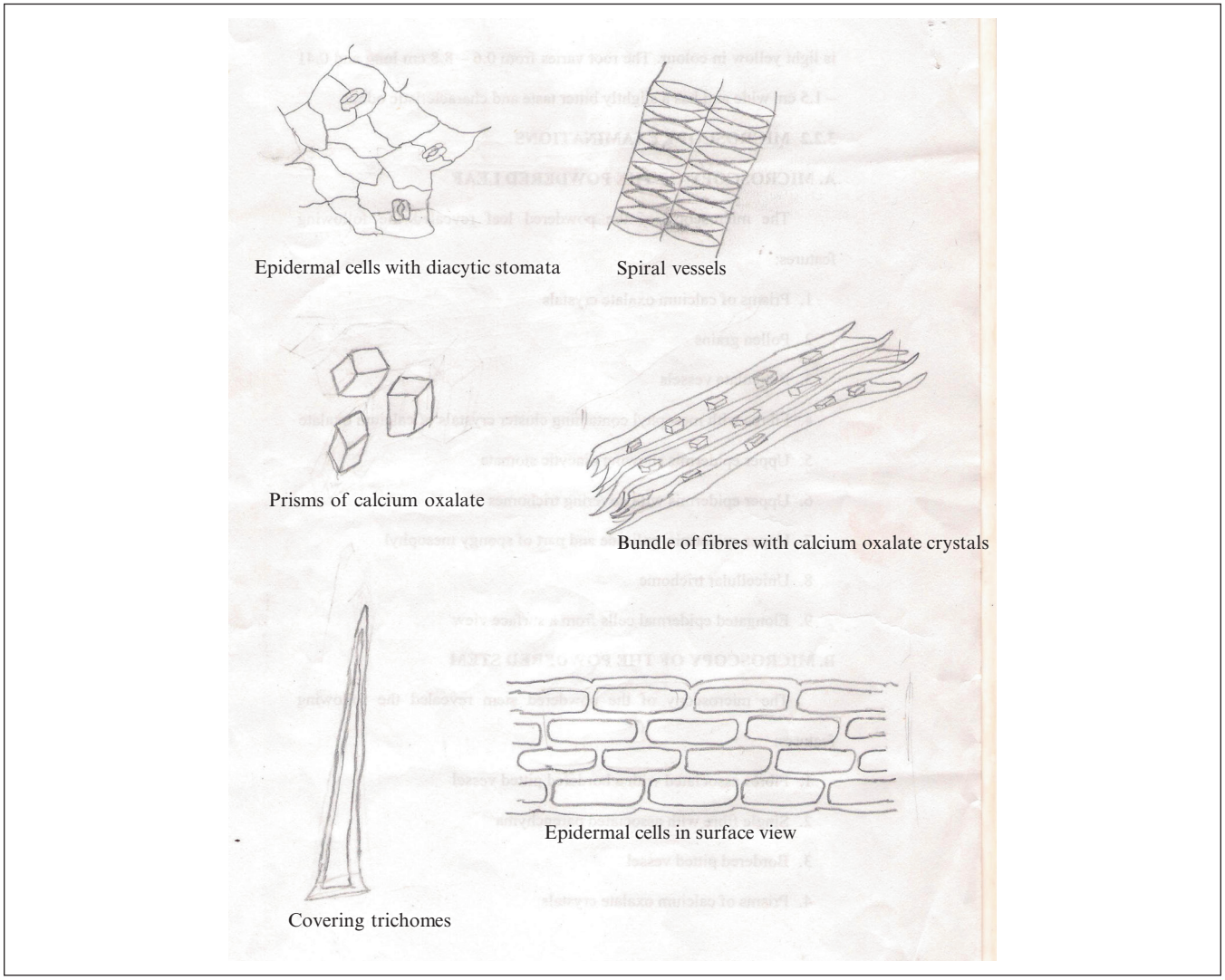

Figure 1: Macroscopical features of the leaf of *Dyshoriste perroteti* Nees

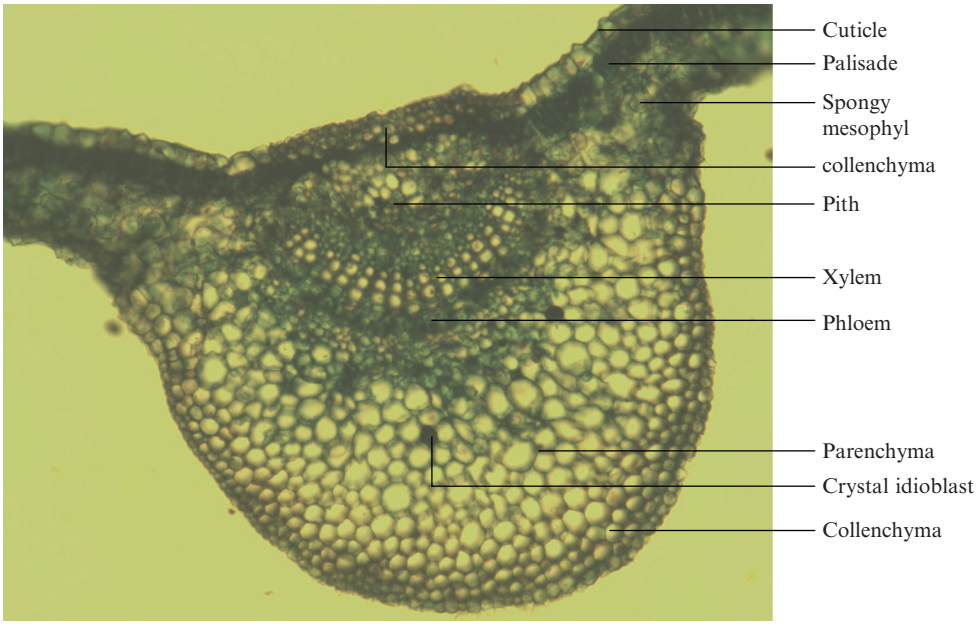

Figure 2: Transverse section showing the midrib of the leaf of *Dyshoriste perrottetii* Nees

fibers moderate, 100-200 μm long wih tapering apex, spiral xylem vessels, prisms of calcium oxalate 25-30 μm in size. The transverse section of the lamina through the midrib (Figure 2) revealed that the is dorsoventral with epidermis covered externally by a wavy cuticle, mesophyll, diacytic stomata and a characteristic collenchyma cells below the epidermis

Phytochemical studies

Phytochemical screening of the leaf powder revealed the presence of alkaloids, fiavonoids, tannins, glycosides, saponins and sterols.

Chemo-microscopical examination

This revealed the presence of chemical constituents in the cell wall and cell of *Dyschoriste perrottetii* (Table 1).

Quantitative leaf microscopy

The results of quantitative microscopy and pharmacognostic standards were presented in Table 2.

Physicochemical standards

The water extractive and alcohol extractive, total ash, acid insoluble ash, water soluble ash and moisture content were shown in Table 3.

DISCUSSION

The macroscopical features of the plant can be used, as its diagnostic parameters. The microscopical features such as the presence of diacytic stomata on both epidermal surfaces, aggregate of calcium carbonate (cystolith), the parenchymatous cells containing prismatic calcium oxalate crystals conformed with major characteristic features of the family Acanthaceace.[10] The chemo-microscopical result indicated the presence of mucilage and tannins. Phytochemical screening reveals the presence alkaloids, fiavonoids, tannins, glycoside, saponnins and sterols. The commonly encountered alkaloid in the Acanthaceace family is the trophan alkaloids, quinazoline found to have

Table 1: Results of chemomicroscopy of the leaf of *Dyschoriste perrottetii* Nees

Test Reagent	Observation	Inference
Chlo-zinc-iodide	Blue to black colour observed on epidermal cells	Cellulose (+)
Ferric chloride solution	Greenish leaves in some parenchyma cells	Tannins (+)
N 50 – Iodine	Blue-black colouration observed on some few grains in parenchyma cells. In transverse section and in powdered leaves.	Starch (+)
Phloroglucinol and conc. HCL	No. red colouration observed in the xylem vessels	Lignin (–)
Ruthenium red	Red colouration observed	Mucilage (+)
80 % H_2SO_4	Crystals of calcium oxalate dissolved	Calcium oxalate crystals (+)

Table 2: Results of quantitative microscopy of the Leaf of *Dyschoriste perrottetii* Nees

Standard	Value
Palisade ratio	8.4 ± 0.11
Stomatal number	23.0 ± 1.32
Upper epidermis	19.9 ± 0.50
Lower epidermis	37.1 ± 0.16
Stomatal index	1.12 ± 1.08
Vein islet	15.0 ± 0.84
Vein termination	13.0 ± 2.06

Values are mean 3 determinations

Table 3: Results of Analytical standards of the leaf of *Dyschoriste perrottetii* Nees

Determination	Values
Moisture content	7.5 ± 1.50
Total ash	17.5 ± 1.92
Acid-insoluble ash	4.0 ± 0.21
Alcohol extractive	13.2 ± 1.55
Water extractive	31.2 ± 0.10

Values are mean 3 determinations

utrotonic.[11] This may be responsible for the use of the plant in easing labour. The presence of tannin and other phenolic compounds which are known to have antimicrobial activity were revealed in the phytochemical analysis and chemomicroscopy. This therefore justifies the use of the plant in the traditional treatment venereal diseases, urinary tract infection and diarrohoea.[5]

The pharmacognostics standards such as moisture content (7.5 % w/w) of the leaf, which is low, showed that there is less chance for microbial degradation of the drug during storage.

CONCLUSION

The results presented in this study could serve as diagnostic parameters for proper identification as well as preparation of a monograph on Dyschoriste *perrottetii* Nees.

ACKNOWLEDGEMENT

The authors thank Department of Pharmacognosy and Environmental Medicine and Department of Botany, University of Nigeria, Nsukka for providing the facilities for the research.

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Medicinal Plants used for Postnatal Care in Malay Traditional Medicine in the Peninsular Malaysia

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ABSTRACT

Malay traditional medicine practice concentrates on the primary healthcare including physical and spiritual aspects of human being. Most traditional practitioners use medicinal plants in the treatment. Hence, the study is aimed to compile preparations and local medicinal plants used traditionally by the women in postnatal care. Five Malay traditional practitioners based in the district of Muar in Johor and two in the district of Kuala Pilah in Negeri Sembilan were interviewed. From the study, information on 23 preparations, consisting of 128 medicinal plants, was successfully compiled. The preparations were categorised as jamu, fresh herbs, eye drop, poultice, medicated talcum powder and bathing solution. The medicinal plants comprised of 52 species belonging to 42 genera and 27 families. Some species were found to occur frequently, such as *Curcuma longa* L., *Zingiber officinale* Roscoe, *Cinnamomum zeylanicum* Blume, *Kaempferia galanga* L., *Piper cubeba* Bojer, *Zingiber cassumunar* Roxb., *Acorus calamus* L., *Piper nigrum* Beyr. ex Kunth, *Alyxia stellata* Roem. & Schult., *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Nigella sativa L.* and *Usnea barbata* Fries. The part of plants utilised in the preparations include rhizomes, fruits/berries, leaves, seeds, barks, flowers, roots, whole plant, gall and bulb. The study provided useful and important information on the diversity of medicinal plants used by different Malay traditional practitioners in postnatal care.

Key words: *medicinal plants, postnatal care, Malay traditional medicine*

INTRODUCTION

The practice of Malay traditional medicine has various influences, for example by the Indonesian, Chinese and Indian traditional medicines, *orang asli* medicine and including those introduced by the Arabs, Persians and Europeans.[1] However, nowadays the practice is mainly dominated by the Arabic Unani medicine and Galenic philosophy. In the context of socioanthropology, structure of the Malay traditional medicine is not fixed and rigid, thus allowing improvements and changes to be made according to suitability and current needs.[2]

The Malay traditional medicine system believes that a person consists of two aspects: (a) physical, that is the body; and (b) spiritual.[3,4] The physical characteristic of a person comprises of four elements (fire, earth, wind and water) and humours (damp, cold, dry and hot).[5] Often a cold condition, due to either consuming "cold" foods and drinks or being in a cold weather, may result to the person building up excessive wind within the body and consequently this will cause the immediate or precipitating illness.[6] The spiritual aspect, on the other hand, constitutes of the mind and soul substance or vital force (*semangat*); thus a person with a loss of *semangat* is said to be vulnerable to the influence of supernatural or evil spirits.[3] The cause of an ailment is often thought to be due to the imbalance of the above mentioned physical elements and/or loss of *semangat*.

Various methods are used in the treatment of illnesses including use of herbal medicines such as spices, medicinal plants and animals; physical treatment such as massage, suction therapy and circumcision; as well as spiritual treatment such as recitation and performing prayers. Medication of physical illness is usually prescribed, of which characteristics must be opposite to those of the ailment.

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DOI: 10.5530/pj.2011.24.4

For example, a "cold" ailment or that caused by excessive wind in the body will be prescribed with a "hot" medicine. Medications containing single or compound medicinal plants may be dispensed in many forms such as powder, capsules, pills, "makjun", medicated oil, simple distillate, decoction, infusion, paste and poultice. Herbal medicines are often used for medicinal purposes and are sometimes self-prescribed for relief of minor illnesses such as colds, fevers, coughs, diarrhea, stomach-aches and headaches. These are also more popularly taken as health supplements for the maintenance of physical fitness and health, restoration of new power and spirit of life, as well as reassuring matrimonial happiness.

In Malay traditional medicine, traditional midwife (*mak bidan*) is an important practitioner owing to her role in treating and advising women on health care and health problems. The fundamental function of a traditional midwife is taking care of a pregnant mother before, during and after childbirth. Frequently, women seek help from the traditional midwife when they have problems associated with the reproductive system. Treatment is often carried out by body massage with either ordinary cooking oil or medicated oil. Massage has been found to be beneficial for relaxation of the body, as well as for relieve of joint and muscle pain and stiffness. Sometimes a mixture of medicinal plants, commonly known as jamu (unprocessed or dried natural materials used for medicinal or health care purposes), is prescribed to the patient. Jamu is traditionally used for the relief of minor illnesses, as health supplements and food supplements, as well in cosmetics. Other preparations made by the traditional midwife include *air selusuh* (medicine for before and during childbirth), *ubat periuk* (medicine for after childbirth), *makjun* (spherical semi-solid preparation), *bedak sejuk* (rice talcum powder), *param* (medicated talcum paste), liniment and hot compression.[1] Most Malay communities believe that special attention and care should be given to a new mother during her confinement period in order to help restore energy and vitality. Jamu is usually given in the morning, followed by body massage for at least three consecutive days. Later, hot compression using a wrapped hot stone is applied onto the abdominal part. A medicated paste is then spread over the stomach and the waist is bound tightly with a long piece of cloth. Additionally, a medicated paste may be applied onto the forehead and face.

Proper documentation of the use of plants in the Malay traditional medicine practice is very limited, such as publications by Ridley,[7] Gimlette,[8,9] Gimlette and Burkill,[10] Gimlette and Thomson,[11] and Burkill,[12] and these are not regularly updated. No formal training of traditional Malay practitioners is currently available. Knowledge and traditional prescriptions are passed from generation to generation merely by word of mouth. Thus, this paper provides preliminary information on plants used traditionally by the women in postnatal care in Malaysia and possible justifications based on previously published traditional uses and scientific data for selected medicinal plants. The objective of the study is to compile information on the type and purpose of preparations, as well as the type and part of medicinal plants. This was done by oral interview of randomly selected Malay traditional medicine practitioners who are based in the districts of Muar in Johor and Kuala Pilah in Negeri Sembilan, in the Peninsular Malaysia.

MATERIALS AND METHODS

Seven Malay traditional medicine practitioners were individually interviewed based on a set of pre-piloted questionnaire. Five of them were based in Muar, Johor and two were in Kuala Pilah, Negeri Sembilan (Figure 1). The practitioners were Hussain, Kalsom, Painah and Salmi from Kampung Parit Medan, Muar, Johor. Zainab was from Kampung Tiong Baru, Muar, Johor. Yah and Ujang were from Kampung Tanjung Jati, Kuala Pilah, Negeri Sembilan.

Information enquired in the questionnaire include: (i) type of preparation, (ii) name of medicinal plant(s), (iii) part of the medicinal plant used, (iv) method of preparation and (v) use of the preparation. The data gathered from the interview was analysed. Most of the names of medicinal plants were given in Malay, therefore, the scientific names were cross-checked with several ethnobotanical references such as Burkill,[13] Zakaria and Mohd,[1] and Mat-Salleh and Latiff.[14] The reported traditional uses of these plants in Malaysia by Burkill[13] were also obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases.[15]

RESULTS AND DISCUSSION

Preparations Used for Postnatal Care in Malay Traditional Medicine

Twenty three preparations consisting of 128 medicinal plants were compiled, as summarised in Table 1. The types of preparations included jamu, *ulam* (fresh herb), eye drops, *tapel* (poultice), *pilis* (medicated talcum paste applied onto the forehead), *param* (medicated talcum paste applied to the whole body) and *mandian* (herbal bath).

In the Malay traditional medicine, jamu is traditionally used in post-partum medication to help improve blood flow, warming and refreshing of the body, speed up contraction of the uterus and tightening of the vagina, encourage bowel movement and prevent vaginal discharge. Jamu often contains a mixture of various medicinal plants and plant parts that is evidenced from this study (Table 1: J1-J8). It is given orally in a form of either herbal pills or hot water

Figure 1: A map of Peninsular Malaysia showing the sites of study

decoction or hot water mixture. The composition of jamu is found to vary according to the Malay traditional practitioners. The finding seems to agree with the statement of Salleh[2] that the practice is not rigid. This is unlike other traditional or complementary practices whereby certain ailment is treated with a specified medicine. In this study, 61 medicinal plants of 35 species are found to be used in the jamu preparations and the most frequently used include *Curcuma longa* L. (in 5 preparations), *Coriandrum sativum* L. (3), *Kaempferia galanga* L. (3), *Parkia roxburghii* G.Don (3), *Quercus infectoria* Oliv. (3) and *Usnea barbata* Fries (3). The applications of *C. longa* in the Malay traditional medicine have been recorded by Burkill[13] for parturition and other ailments related to afterbirth such as amenorrhea, diuretic, lactagogue, swelling, tonic, urogenital problems and wound healing (Table 2). Previous studies on *C. longa* have revealed that the extracts have antidepressant,[16] hypotensive and vasorelaxant[17] effects in vivo. Curcumin isolated from *C. longa* rhizome has been shown to exhibit activity against various pro-inflammatory diseases such as cancer, diseases of the heart, lung, liver and skin, neurodegenerative and endocrine disorders, infectious diseases and others,[18] whereas *ar*-turmerone is a potent inhibitor for collagen-induced platelet aggregation[19] and an immunomodulator.[20]

Poultice, medicated talcum paste applied onto the forehead, medicated talcum paste applied to the body parts and herbal bath are used as external preparations. In this study, the

Table 1: Plant species, local Malay name, parts of plants and applications of medicinal plants used in postnatal care by the Malay traditional medicine practitioners in Muar, Johor and Kuala Pilah, Negeri Sembilan

No.[a]	Species	Malay Name	Part Used	Applications
J1	*Acorus calamus* L.	Jerangau	Rhizome	Hot water mixture of the ground plant materials is given to improve blood circulation, to make the body feel warm, to encourage contraction of the uterus, to expel wind, to prevent fit and as a laxative.
	Alpinia conchigera Griff.	Lengkuas padang	Rhizome	
	Alyxia stellata Roem. & Schult.	Pulasari	Bark	
	Carum carvi L.	Jemuju	Fruit	
	Cinnamomum zeylanicum Blume	Kayu manis	Stem bark	
	Coriandrum sativum L.	Ketumbar	Fruit	
	Curcuma longa L.	Kunyit	Rhizome	
	Elaeocarpus grandiflorus Sm.	Anyang-anyang	Bark	
	Foeniculum vulgare Mill.	Adas pedas	Fruit	
	Illicium tenuifolium (Ridl.) A.C.Sm.	Bunga lawang bukit	Flower	
	Kaempferia galanga L.	Cekur	Leaf	
	Nigella sativa L.	Jintan hitam	Seed	
	Oroxylum indicum (L.) Benth. ex Kurz	Bonglai	Fruit	
	Parkia roxburghii G.Don	Kedaung	Seed	
	Peucedanum japonicum Thunb.	Ganti	Rhizome	
	Piper cubeba Bojer	Kemungkus	Berry	
	Piper nigrum Beyr. ex Kunth	Lada putih & hitam	Fruit	
	Piper retrofractum Vahl	Cabai sirih	Fruit	
	Quercus infectoria Oliv.	Manjakani	Gall	
	Rheum officinale Baill.	Kelembak	Root	
	Saussurea lappa C.B.Clarke	Pucuk	Root	
	Usnea barbata Fries	Kayu angin	Whole plant	
	Zingiber officinale Roscoe	Halia	Rhizome	
J2	*Coriandrum sativum* L.	Ketumbar	Fruit	Hot water mixture of ground plant materials is given to improve blood circulation, to regain body strength, to expel wind, to ease muscular and joint pain, as well as to ease abdominal discomfort.
	Curcuma longa L.	Kunyit	Rhizome	
	Parkia roxburghii G.Don	Kedaung	Seed	
	Piper nigrum Beyr. ex Kunth	Lada putih	Fruit	
	Usnea barbata Fries	Kayu angin	Whole plant	
	Zingiber aromaticum Valeton	Lempoyang	Rhizome	
	Zingiber officinale Roscoe	Halia	Rhizome	
J3	*Alyxia stellata* Roem. & Schult.	Pulasari	Bark	Hot water mixture of ground plant materials is given to regain body strength, to prevent bad body odour, to expel wind, to encourage contraction of the uterus, to encourage wound healing, to reduce bleeding, to stimulate lactation and as a contraceptive.
	Carum carvi L.	Jemuju	Fruit	
	Cinnamomum zeylanicum Blume	Kayu manis	Stem bark	
	Coriandrum sativum L.	Ketumbar	Fruit	
	Eurycoma longifolia Jack	Kayu pasak bumi	Root	
	Foeniculum vulgare Mill.	Adas pedas	Fruit	
	Illicium tenuifolium (Ridl.) A.C.Sm.	Bunga lawang bukit	Flower	
	Kaempferia galanga L.	Cekur	Leaf	
	Litsea odorifera Valeton	Terawas	Flower	
	Nigella sativa L.	Jintan hitam	Seed	
	Parameria polyneura Hook.f.	Kayu rapat	Stem bark	
	Parkia roxburghii G.Don	Kedaung	Seed	
	Piper cubeba Bojer	Kemungkus	Berry	
	Quercus infectoria Oliv.	Manjakani	Gall	
	Usnea barbata Fries	Kayu angin	Whole plant	
J4	*Curcuma longa* L.	Kunyit	Rhizome	Juice mixture with dark brown sugar and salt is given for slimming.
	Tamarindus indica L.	Asam jawa	Fruit	
J5	*Quercus infectoria* Oliv.	Manjakani	Gall	Ground dried gall is given to encourage contraction of the uterus, to regain body strength, to treat vaginal discharge and to treat abdominal discomfort.
J6	*Rourea humilis* Blume	Akar kayu mengecut	Root	Its water decoction is given to encourage contraction of the uterus.
J7	*Ananas comosus* (L.) Merr.	Nanas	Young leaf	Its juice mixture is given to stimulate lactation.
	Curcuma longa L.	Kunyit	Rhizome	
	Curcuma heyneana Valeton & Zijp	Temu giring	Rhizome	
	Curcuma mangga Valeton & Zijp	Temu pauh	Rhizome	
	Curcuma xanthorrhiza D.Dietr.	Temu lawak	Rhizome	
	Kaempferia galanga L.	Cekur	Leaf	
	Musa acuminata Colla	Pisang kapas	Fruit	
	Tamarindus indica L.	Asam jawa	Fruit	
	Zingiber aromaticum Valeton	Lempoyang	Rhizome	

No.[a]	Species	Malay Name	Part Used	Applications
J8	*Acorus calamus* L.	Jerangau	Rhizome	Hot water mixture of ground plant materials with tamarind and dark brown sugar is given to stimulate lactation and to prevent bad odour of the breasts.
	Curcuma longa L.	Kunyit	Rhizome	
	Zingiber cassumunar Roxb.	Bongelai	Rhizome	
U9	*Centella asiatica* (L.) Urb.	Pegaga	Leaf	Fresh leaf is eaten to stimulate lactation.
U10	Zingiber officinale Roscoe	Halia	Rhizome	Fried rhizome is eaten to make the body feel warm.
E11	*Piper cubeba* Bojer	Kemungkus	Berry	Its juice is applied into the eyes to improve eyesight.
T12	*Tamarindus indica* L.	Asam jawa	Fruit	Mixture of its juice and lime is applied onto the abdomen for slimming and to encourage contraction of the uterus.
T13	*Zingiber officinale* Roscoe	Halia	Rhizome	
T14	*Citrus aurantifolia* Swingle	Limau nipis	Fruit	
P15	*Acorus calamus* L.	Jerangau	Rhizome	Mixture of the ground plant materials and water is applied onto the forehead to help improve eyesight.
	Alyxia stellata Roem. & Schult.	Pulasari	Bark	
	Cinnamomum zeylanicum Blume	Kayu manis	Stem bark	
	Eugenia aromatica Kuntze	Cengkih	Flower bud	
	Foeniculum vulgare Mill.	Adas pedas	Fruit	
	Illicium tenuifolium (Ridl.) A.C.Sm.	Bunga lawang bukit	Flower	
	Nigella sativa L.	Jintan hitam	Seed	
	Peucedanum japonicum Thunb.	Ganti	Rhizome	
	Piper cubeba Bojer	Kemungkus	Berry	
	Rheum officinale Baill.	Kelembak	Root	
	Sesbania grandiflora (L.) Pers.	Turi	Leaf	
	Zingiber cassumunar Roxb.	Bongelai	Rhizome	
P16	*Cinnamomum zeylanicum* Blume	Kayu manis	Stem bark	Mixture of the ground plant materials and water is applied onto the forehead to help improve eyesight.
	Entada phaseoloides (L.) Merr.	Sintok	Seed	
	Eugenia aromatica Kuntze	Cengkih	Flower bud	
	Nigella sativa L.	Jintan hitam	Seed	
	Piper cubeba Bojer	Kemungkus	Berry	
	Piper nigrum Beyr. ex Kunth	Lada putih	Fruit	
P17	*Allium sativum* L.	Bawang putih	Bulb	Mixture of the ground plant materials and water is applied onto the forehead to help improve eyesight and to treat headache.
	Cinnamomum zeylanicum Blume	Kayu manis	Stem bark	
P18	*Acorus calamus* L.	Jerangau	Rhizome	Mixture of the ground plant materials and water is applied onto the forehead to help improve eyesight and to freshen the body.
	Cinnamomum zeylanicum Blume	Kayu manis	Stem bark	
	Eugenia aromatica Kuntze	Cengkih	Flower bud	
	Piper cubeba Bojer	Kemungkus	Berry	
	Piper nigrum Beyr. ex Kunth	Lada hitam	Fruit	
	Sesbania grandiflora (L.) Pers.	Turi	Leaf	
R19	*Alyxia stellata* Roem. & Schult.	Pulasari	Seed	Mixture of the ground plant materials and a little water is applied onto the face to freshen the body.
	Foeniculum vulgare Mill.	Adas pedas	Fruit	
	Kaempferia galanga L.	Cekur	Leaf	
	Oryza sativa L	Beras	Seed	
	Peucedanum japonicum Thunb.	Ganti	Rhizome	
	Zingiber officinale Roscoe	Halia	Rhizome	
	Zingiber cassumunar Roxb.	Bongelai	Rhizome	
R20	*Curcuma longa* L.	Kunyit	Rhizome	Mixture of the ground plant materials and a little water is applied onto the face to freshen the body and to expel wind.
	Kaempferia galanga L.	Cekur	Leaf	
	Oryza sativa L	Beras	Seed	
	Usnea barbata Fries	Kayu angin	Whole plant	
	Zingiber officinale Roscoe	Halia	Rhizome	
R21	*Curcuma longa* L.	Kunyit	Rhizome	Mixture of the ground plant materials and a little water is used to massage the body and to make the body feel warm.
	Kaempferia galanga L.	Cekur	Rhizome	
	Oryza sativa L	Beras	Seed	
	Piper nigrum Beyr. ex Kunth	Lada putih	Fruit	
	Vetiveria zizanioides Stapf	Larasetu	Root Rhizome	
	Zingiber cassumunar Roxb.	Bongelai	Rhizome	
	Zingiber officinale Roscoe	Halia		
M22	*Acorus calamus* L.	Jerangau	Leaf	Mixture of the water decoction and plenty of water is used for bathing in order to remove bad body odour and to freshen the body.
	Alpinia galanga Willd.	Lengkuas	Leaf	
	Carica papaya L.	Betik	Leaf	
	Citrus aurantifolia Swingle	Limau nipis	Leaf	
	Coriandrum sativum L.	Ketumbar	Leaf	
	Curcuma longa L.	Kunyit	Leaf	

(Continued)

Table 1: *Continued*

No.[a]	Species	Malay Name	Part Used	Applications
	Curcuma xanthorrhiza D.Dietr.	Temu lawak	Leaf	
	Cymbopogon citratus Stapf	Serai makan	Leaf	
	Piper betle L.	Sireh	Leaf	
	Psidium guajava L.	Jambu batu	Leaf	
	Zingiber cassumunar Roxb.	Bongelai	Leaf	
M23	*Alpinia galanga* Willd.	Lengkuas	Leaf	Mixture of the water decoction and plenty of water is used for bathing in order to remove bad body odour and to make the body feel warm.
	Coleus blumei Benth.	Ati-ati	Leaf	
	Cymbopogon nardus (L.) Rendle	Serai wangi	Leaf	
	Datura fastuosa L.	Kecubung	Leaf	
	Pandanus odorus Ridl.	Pandan	Leaf	
	Zingiber cassumunar Roxb.	Bongelai	Leaf	

[a]Indicates the type of preparation, that is, J=Jamu, U=*Ulam* (fresh herbs), E=*Titisan Mata* (eye drops), T=*Tapel* (poultice), P=*Pilis* (medicated talcum powder applied onto the forehead), R=*Param* (medicated talcum powder applied to the body), M=*Mandian* (bathing solution). Definition of each preparation is described in the text.

Table 2: Families of the medicinal plant species used medicinal plants used after childbirth by the Malay traditional medicine practitioners in Muar, Johor and Kuala Pilah, Negeri Sembilan and the reported traditional uses

Family	Species	Reported Traditional Uses by Burkill (1966)
Acoraceae	*Acorus calamus* L.	Diarrhea, odontosis, parturition, pediculifuge, splenomegaly, venereal, vermifuge.
Apiaceae	*Carum carvi* L.	–
	Centella asiatica (L.) Urb.	–
	Coriandrum sativum L.	Cough, fever, nausea, ophthalmia, rheumatism.
	Foeniculum vulgare Mill.	Abdomen, dermatosis, gastralgia, hepatosis, rheumatism.
	Peucedanum japonicum Thunb.	Constipation, fever, giddiness, miscarriage, parturition, sapraemia, smallpox.
Apocynaceae	*Alyxia stellata* Roem. & Schult.	–
	Parameria polyneura Hook.f.	Parturition, tonic, toothblack, uteromegaly.
Asteraceae	*Saussurea lappa* C.B.Clarke	–
Bignonaceae	*Oroxylum indicum* (L.) Benth. ex Kurz	Ache (stomach and tooth), cholera, diarrhea, dysentery, enterosis, fever, gastrosis, parturition, rheumatism, splenomegaly, swelling, vertigo.
Bromeliaceae	*Ananas comosus* (L.) Merr.	Abortifacient, diphtheria, diuretic, emmenagogue, gonorrhoea, vermifuge.
Caricaceae	*Carica papaya* L.	Abortifacient, arthritis, asthma, boil, colic, dysuria, fever, fumitory, gravel, vermifuge.
Connaraceae	*Rourea humilis* Blume	–
Elaeocarpaceae	*Elaeocarpus grandiflorus* Sm.	Sore, tonic.
Fabaceae	*Entada phaseoloides* (L.) Merr.	Abdomen, cosmetic, enterosis, hematochezia, parturition, piscicide, shampoo, watervine, wound.
	Parkia roxburghii G.Don	–
	Sesbania grandiflora (L.) Pers.	Aperient, bruise, cosmetic, diarrhea, dysentery, edema, emetic, enterosis, gastrosis, glossitis, leucorrhea, ophthalmia, scabies, sore (throat), sprain, sprue, stomatitis, thrush, tonic, tonsillitis.
	Tamarindus indica L.	Abortive, boil, conjunctivitis, cosmetic, dermatosis, fever, itch, mucusitis, pimple, rheumatism, sore, sting (caterpillar), thrush, wound.
Fagaceae	*Quercus infectoria* Oliv.	–
Illiciaceae	*Illicium tenuifolium* (Ridl.) A.C.Sm.	–
Lamiaceae	*Coleus blumei* Benth.	Cachexia, dyspepsia, ophthalmia
Lauraceae	*Cinnamomum zeylanicum* Blume	–
	Litsea odorifera Valeton	Lactagogue
Liliaceae	*Allium sativum* L.	Vermifuge
Musaceae	*Musa acuminata* Colla	–
Myrtaceae	*Eugenia aromatica* Kuntze	Ache (head and tooth), colic, parturition, tonic, vaginamegaly.
	Psidium guajava L.	Ache (stomach), dermatosis, diarrhea, emmenagogue, epilepsy, hysteria, leucorrhea, swelling, vermifuge.
Pandanaceae	*Pandanus odorus* Ridl.	Anemia, cosmetic, gonorrhoea, measles, parturition, sapraemia, syphilis.
Parmeliaceae	*Usnea barbata* Fries	–

Family	Species	Reported Traditional Uses by Burkill (1966)
Piperaceae	*Piper betle* L.	Abscess, ache (tooth), antiseptic, asthma, cough, earache, epistaxis, gingivitis, gonorrhoea, halitosis, hoarseness, itch, lactafuge, leucorrhea, mucositis, otosis, parturition, pimple, sore, stimulant.
	Piper cubeba Bojer	Aphrodisiac, diuretic, dysentery, dyspepsia, enterosis, gonorrhoea, panacea, rheumatism, stimulant.
	Piper nigrum Beyr. ex Kunth	Abortifacient, ache (head), cholera, colic, gonorrhoea.
	Piper retrofractum Vahl	Cramps, dyspepsia, hepatosis, osteosis, weakness.
Poaceae	*Cymbopogon citratus* Stapf	Ache (tooth), diaphoretic, diuretic, dyspepsia, emmenagogue, gingivitis, neuritis, rheumatism, sprain.
	Cymbopogon nardus (L.) Rendle	Dyspepsia, emmenagogue.
	Oryza sativa L.	–
	Vetiveria zizanioides Stapf	Cosmetic, parturition.
Polygonaceae	*Rheum officinale* Baill.	Cosmetic, freckles, fumitory, purgative, tonic.
Ranunculaceae	*Nigella sativa* L.	–
Rutaceae	*Citrus aurantifolia* Swingle	Ache (head and stomach), cough, dermatosis, dysentery, gonorrhoea, neuralgia, yaws.
Simaroubaceae	*Eurycoma longifolia* Jack	Ache (head), fever, malaria, parturition, smallpox, sore, syphilis, wound.
Solanaceae	*Datura fastuosa* L.	–
Zingiberaceae	*Alpinia conchigera* Griff.	–
	Alpinia galanga Willd.	–
	Curcuma heyneana Valeton & Zijp	Deodorant, obesity, wound.
	Curcuma longa L.	Abscess, amenorrhea, cold, conjunctivitis, cosmetic, diarrhea, diuretic, dysentery, gonorrhoea, gravel, hepatosis, jaundice, lactagogue, parturition, pyuria, scabies, sore, swelling, tonic, urogenital, wound.
	Curcuma mangga Valeton & Zijp	Fever, stomachic.
	Curcuma xanthorrhiza D.Dietr.	Amenorrhea, choleretic, constipation, dyspepsia, emmenagogue, gallstones, hepatosis, parturition, rheumatism.
	Kaempferia galanga L.	Abdomen, cosmetic, cough, fever, mastitis, ophthalmia, otosis, rheumatism, sore (throat), swelling.
	Zingiber aromaticum Valeton	Bilious, chlorosis, cholecystosis, gout, parturition, pertussis, tonic.
	Zingiber cassumunar Roxb.	Abdomen, ache (head), ache (stomach), anodyne, colic, constipation, cosmetic, cramps, fever, flatulence, gonorrhoea, jaundice, malaria, numbness, parturition, vermifuge.
	Zingiber officinale Roscoe	Abortive, ache (back, head and stomach), ague, colic, congestion, cosmetic, cough, dyspepsia, fever, gingivosis, gynecology, hepatosis, infection, panacea, parturition, puerperium, rheumatism, rhinosis, sore, swelling, syphilis, tonic.

(–) Information not available in Burkill (1966).

poultice is made up of a mixture of lime (*Citrus aurantifolia* Swingle) and juice of either *Tamarindus indica* L. or *Zingiber officinale* Roscoe and is applied onto a mother's abdomen. This is said to encourage contraction of the uterus and slimming of the abdominal part (Table 1: T12-T14). The latter traditional use could be associated with the findings that methanol and ethyl acetate extracts of *Z. officinale* reduce abdominal fat deposition in vivo.[21] Additionally, the use of *Z. officinale* in this preparation is also in accordance to the reported traditional use of *Z. officinale* in parturition and during puerperium[13] (Table 2). Furthermore, the Sundaneses in West Java, Indonesia have also used *T. indica* fruit in their post-partum remedies.[22]

The medicated talcum paste, *pilis*, applied onto a mother's forehead is traditionally believed to help improve poor vision and to treat headache after childbirth. Fifteen species in 4 preparations have been compiled in this study. *Cinnamomum zeylanicum* Blume (in 4 preparations), *Eugenia aromatica* Kuntze (3) and *Piper cubeba* Bojer (3) are three most commonly utilised in the *pilis* (Table 1: P15-P18). Only *E. aromatica* has been reported to be used traditionally in the treatment of headache[13] (Table 2). Eugenol, which is the active ingredient of *E. aromatica,* is found to inhibit monoamine oxidase A in vitro and has antidepressant activity in vivo.[23]

Traditionally, medicated talcum paste *param* used for massaging or applied to the entire body is considered to help regain body figure, expel wind, eliminate stretch marks and smoothen the skin. In *param*, *K. galanga* (in 3 preparations), *Oryza sativa* L. (3) and *Z. officinale* (3) are most popular ingredients (Table 1: R19-21). It has been revealed that methanolic extract of *K. galanga* has antinociceptive effect in vivo[24] and the hexane extract has sedative property.[25] In addition, *O. sativa* has been used in the traditional

preparation of the Sundaneses in West Java, Indonesia in the treatment of dermatitis.[22] *O. sativa* could also be used as an excipient in the powdered mixture due to the high content of starchy materials for easy and uniform application of the paste.

In the Malay traditional medicine, new mothers are encouraged to bathe with water-boiled leaves or roots. In this study, 17 medicinal plants in 2 preparations were compiled and *Alpinia galanga* Willd. (in 2 preparations), *Zingiber cassumunar* Roxb. (2) and *Cymbopogon spp.* (2) are found to be common ingredients of the solutions (Table 1: M22-M23). The use of aromatic herbs is believed to help remove bad body odour, to freshen the body, to make the body feel warm, to help expel wind from the body and to smoothen the skin. The applications on the skin could be supported by the fact that essential oil of *Z. cassumunar* is active against dermatological infections caused by bacteria, dermatophytes and yeasts[26] and *C. citratus* oil has potent *in vitro* antifungal effect against *Candida* spp.[27] In addition, *A. galanga* extract has protective effects on UVA-dependent melanogenesis.[28]

In this study, it is found that fresh herbs (Table 1: U9-U10) are consumed, for example, leaf of *Centella asiatica* (L.) Urb. for stimulation of breast milk and rhizome of *Z. officinale* for warming of the body. Although there is no scientific evidence that *C. asiatica* is galactagogue, interestingly its water extract has been shown to enhance learning and memory of mice during the postnatal developmental stage.[29] Additionally, the methanolic extract of *C. asiatica* has antioxidant activity[30] and the asiaticoside has wound healing effects.[31] One of the practitioners also mentioned the use of *P. cubeba* juice eye drop to improve poor eyesight of a mother after childbirth. The use of herbal eye drop in postnatal treatment is not a common practice. However, methanolic extract of *P. cubeba* has been shown to exhibit anti-inflammatory activity.[32]

Most of the medicinal plants used in the preparations obtained from the study are believed to be "heaty" components so as to complement the "cold" condition after childbirth. This probably explains why most preparations are meant to improve blood circulation, to make the body feels warm, to expel wind, to ease abdominal discomfort and cramps, to ease muscular and joint pain, to stimulate lactation and to act as a laxative and a contraceptive. In fact, the external preparations utilise mainly aromatic medicinal plants, especially the Zingiberaceae species. In aromatherapy, essential oils of *C. zeylanicum, E. aromatica* and *Z. officinale* have been used to ease emotional and mental fatigue. Thus, the use of these aromatic species could help the new mothers to reduce anxiety, reduce stress and improve mood after childbirth.[33]

Medicinal Plants Used for Postnatal Care in Malay Traditional Medicine

In this study, as many as 52 species of medicinal plants from 42 genera are found to be used in the 23 post-partum traditional preparations. These species belong to 27 families, that is, Zingiberaceae (10 species), Apiaceae (5), Fabaceae (4), Piperaceae (4), Poaceae (4), Apocynaceae (2), Lauraceae (2), Myrtaceae (2), Acoraceae (1), Asteraceae (1), Bignonaceae (1), Bromeliaceae (1), Caricaceae (1), Connaraceae (1), Elaeocarpaceae (1), Fagaceae (1) Illiciaceae (1), Lamiaceae (1), Liliaceae (1), Musacea (1) Pandanaceae (1), Parmeliaceae (1) Polygonaceae (1), Ranunculaceae (1), Rutaceae (1), Simaroubaceae (1) and Solanaceae (1) (Table 2).

Some medicinal plants are found to be commonly utilised in the preparations such as *Curcuma longa* L. (7 preparations), *Z. officinale* (7), *C. zeylanicum* (6), *K. galanga* (6), *P. cubeba* (6), *Z. cassumunar* (6), *Acorus calamus* L. (5), *Piper nigrum* Beyr. ex Kunth (5), *Alyxia stellata* Roem. & Schult. (4), *C. sativum* (4), *Foeniculum vulgare* Mill. (4), *Nigella sativa L.* (4) and *U. barbata* (4). It is anticipated that Malay traditional medicine practitioners make use of the easily accessible and inexpensive herbs and spices such as turmeric (*C. longa*), ginger (*Z. officinale*), aromatic ginger (*K. galanga*), cassumunar (*Z. cassumunar*), cinnamon (*C. zeylanicum*), white and black pepper (*P. nigrum*), coriander (*C. sativum*) and sweet fennel (*F. vulgare*) in their preparations. These can be either collected from their home gardens or the neighbouring forests, or purchased from retail stores or local herbal suppliers. However, the use of black seed or black cumin (*N. sativa*) that is not grown locally is rather surprising but could suggest the influence of Prophetic Medicine in the Malay traditional medicine because of the belief that black cumin is a remedy for every disease except death.[34] Besides, several pharmacological properties of *N. sativa* have been reported including CNS-depressant,[35] anti-inflammatory and analgesic,[36] hypotensive, anti-nociceptive, uricosuric, choleretic, anti-fertility, anti-diabetic and anti-histaminic,[37] as well as anti-oxidant, anti-microbial, antitumor and immunomodulatory properties.[38]

Parts of the medicinal plants frequently utilised in the preparations are rhizomes (25%), fruits/berries (22%) and leaves (19%); followed by seeds (9%), barks (9%), flowers (5%), roots (5%), whole plant (3%), gall (2%) and bulb (1%) (Figure 2). Most of the preparations use rhizomes of the Zingiberaceae species; whereas the leaves are mainly used in the bathing preparations. It is also interesting to learn that different parts of a plant are used for different preparations and purposes. For example, the *Z. cassumunar* rhizome is used as internal jamu preparation whereas the leaves are used in the external herbal bath. Frequency of the use of plant parts found in this study is different from the previously reported ethnobotanical studies, whereby

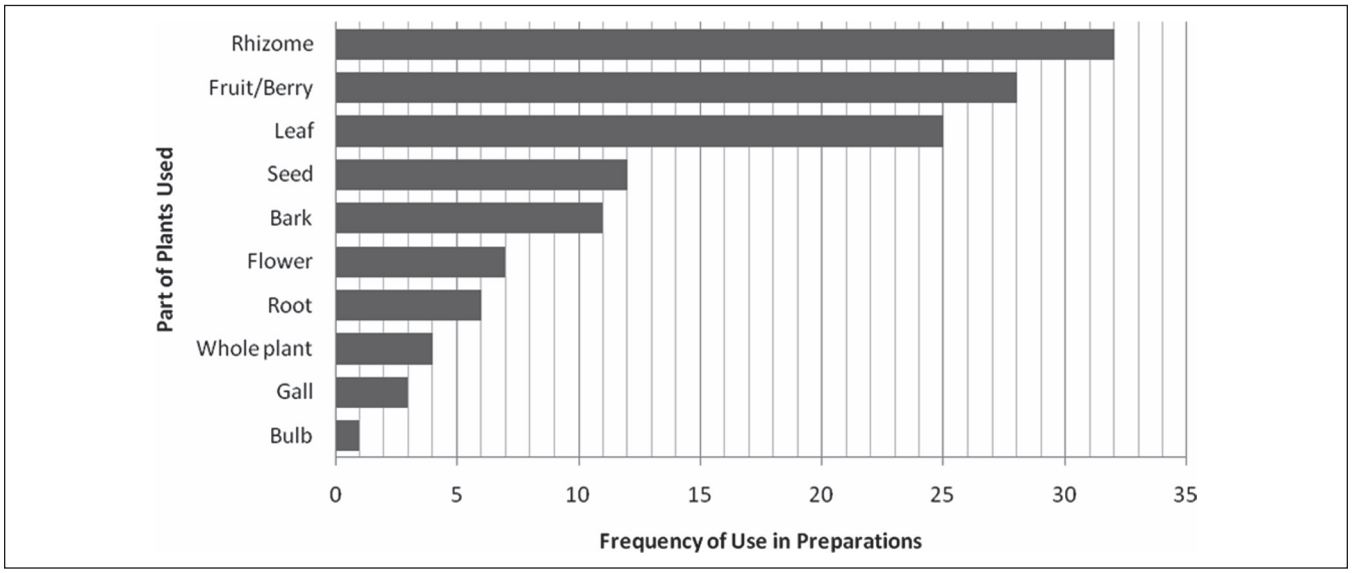

Figure 2: Frequency of plant parts used in the preparations of the Malay traditional medicine for postnatal care

roots and leaves are the most commonly utilised plant parts in the remedies.[39,40] This may exemplify the diversity of traditional practices based on cultural differences.

CONCLUSION

In conclusion, the study has successfully gathered information on the 23 preparations containing 128 medicinal plants used for postnatal care in the Malay traditional medicine at Muar, Johor and Kuala Pilah, Negeri Sembilan. Diverse types of species and parts of plants were used by different practitioners. These are traditionally prepared in various forms, either freshly prepared or dried; powdered or extracted; in a single or compound composition and either taken internally or used externally. Similar studies should be carried out elsewhere in order to compile more data on the use of medicinal plants in the Malay traditional medicine.

ACKNOWLEDGEMENTS

The authors would like to thank the Malay traditional practitioners who were willing to contribute to the study and to the development of knowledge on medicinal plants of Malaysia.

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Biochemical Profiling and Total Flavonoids Contents of Leaves Crude Extract of Endemic Medicinal Plant *Corydyline terminalis* L. Kunth

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ABSTRACT

Now a day the importance of medicinal plants has been increasing both for pharmaceutical industry and traditional users. Most of the countries either it is developing and under developing mostly rely on traditional medicines. This herbal or traditional medicine involves the use of different type organic extracts or the bioactive pure chemical constituents. This type of biochemical investigation provides the health application at minimum cost. This survey such as ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicine. Keeping this view in mind, the present study is carried out in *Corydyline terminalis* L. Kunth leaves collected from rainforest area at Sabah state, Malaysia. This plant has several beneficial properties. The powder leaves of *Corydyline terminalis* L. Kunth was extracted with organic solvent such as hexane, ethyl acetate, chloroform, butanol and methanol. The total phenolic contents of the extracts was determined by Willet method with modification. The results for total flavonoids content of the extracts as caffeic acid equivalents were found to be highest in hexane extract (68.02%) followed by ethyl acetate (61.50%), methanol (39.27%), butanol (19.08%) and chloroform (15.75%). Based on these results it can be suggested that the biochemical properties of the leaves for curing various ailments.

Key words: Biochemical screening, Flavonoids content, Soxhlet extraction, Organic extracts, *Corydyline terminalis* L. Kunth.

INTRODUCTION

Ancient man is well known for their knowledge of utilizing vast variety of drugs for millennia. The crude form of many traditionally used herbs and plants are known to be very useful based on current knowledge. There are at least 121 chemical substances of known structure are still extracted from plants that are useful as drugs around the globe.[1] The World Health Organization (WHO) estimates that more than 4 billion people, 80% of the total world population, presently medication herbal medicine for some aspect of primary health care. In the third world countries, herbal medicine is a major component or ingredient in all indigenous peoples' traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. WHO had noted that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. The major pharmaceutical companies in the world are presently conducting extensive research on plant materials which are gathered from the rain forests and other places for their potential medicinal value.

Today, the U.S. Pharmacopoeia, with its reliance on herbal compounds, has been all but forgotten. The *Physician's Desk Reference*, an extensive listing of chemically manufactured drugs is where most of the modern physicians rely on. It is important to note that each entry in this enormous volume, in addition to specifying the chemical compound and actions of a particular drug, also includes an extensive list of contraindications and possible side effects. Rather than using a whole plant as different types of organic extracts, pharmacologists identify, isolate, extract, and synthesize individual components, thus capturing the active properties.

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DOI: 10.5530/pj.2011.24.5

This can create problems, however, in addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs as traditional medicine can have powerful effects. They should not be taken lightly. Now a day the suggestions to practitioner for herbal treatments in this book are not intended to substitute for consultation with a qualified health care, but rather to support and assist you in understanding and working with your physician's advice.

Chemical substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. Some medicinal herb used in 'Traditional Chinese Medicine' for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

There are so many groups or families of phytochemicals and they help or accelerate to the human body in several ways. Chemical constituents inside the plants may be protecting human body from a host of diseases. Chemical constituents are non-nutritive plant bioactive chemicals that have protective or disease preventive properties. Plant produces itself these bioactive chemicals to protect itself but recent research demonstrates that many chemical constituents can protect humans against diseases. There are so many groups of bioactive chemicals in fruits, vegetables and herbs and each works differently.

So many possible ways to fight or kill these diseases is to improve our body's antioxidant defenses. Comparatively high consumption of vegetables and fruits has been associated with a lowered incidence of such degenerative and incurable diseases.[2] Fruits constituents also help to improve health in other ways. Due to active chemical constituent of fruit juice, can also be taken to alleviate sore throat and seasickness. The functional bioactivity of a plant organic extract, in general, depends upon the presence of compounds such as polyphenols, carotenoids, terpenoids and chlorophyll.[3] Plants also can contribute in this area primarily due to the antioxidant activity of phenolic and flavonoids compounds.[4-8]

By the researcher several studies have done and revealed that the antioxidant capacity may be from compounds inside the plants samples such as flavonoids, isoflavones, flavones, anthocyanins, catechins and other phenolics.[9-11] Various curable and incurable diseases has been linked to the oxidative stress,[9-11] but the food industry has been concerned with issues such as rancidity and oxidative spoilage of foodstuffs.[12] The oxidation for enzyme as well as auto oxidation of amino acid or lipids during storage and processing is the major reaction responsible for the deterioration in food quality affecting the colour, flavour, texture and nutritive value of the foods. They are oftenly added antioxidants to the foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation step leading to the termination of the reaction and a delay in the oxidation process.

Corydyline terminalis L. Kunth, Asparagaceae (monocotyledonous flowering plant) is one of the popular traditional plant used extensively as remedy in Southeast Asia for the treatment of wide range of diseases. Cordylines are known to the world by many names and are crowned as "King of tropical foliage". *Corydyline terminalis* L. Kunth is a shrub plant, with woody stem that can reach a height of 6 metres. This plant which is native to tropical Asia, Australia and New Zealand does not have many branches and its roots can swell to form starch extract, usually occurs in root plants. The name *Cordyline* comes from the Greek word *kordyle*, meaning "club," a reference to the enlarged underground stems or rhizomes.[13] The leaves are simple and in an arrangement of twisted leaves, long, oval or in shape of spear as long as 45 cm and between 7.5-15 cm in width. The stalk consists of long and flat leaves. Consisting of leaves of various colour; red, pink, purplish strips and also with borders of whitish leaves it is reaching high popularity among gardeners, landscapers and collectors alike. The flowers grow in a bunch which is in medium size up to 70 cm long, yellow or purple in colour. Meanwhile the fruits form in ball shapes, reddish in colour with diameter of 2 cm.

In Malaysia, especially Sabah, Kadazandusun's ethnic use *Corydyline terminalis* L. Kunth as medicinal use for curing cough, bloody cough, dysentery, high fever, difficulties in urine, bloody urine, kidney diseases, Tibiae, headache and inflammation in the digestive tract.[14] Furthermore, it is also can also be used to heal scurf and joint pain.[14] People also used *Corydyline terminalis* L. Kunth as popular ornamental plant, with numerous cultivars available, many of them selected for green or reddish or purple foliage despite its variety of medicinal uses. This species is usually planted by the stem or leafy shoots. Some of the villagers plant it by a local method name 'tut'. It is easily planted where the stem part easily grows into a new plant. It is convenient to be planted in pots and on the soil individually, in rows or in groups. This is the first time a research work is being carried out on this tropical traditional medicinal plant. During

our study on the biologically active constituents of this plant, we examined the biochemical screening of the leaves of *Corydyline terminalis* L. Kunth widely used in Sabah community, Malaysia. Hence, the aim of this present study has been made to investigate the biochemical screening and total flavonoids contents of the leaves crude extracts of *Corydyline terminalis* L. Kunth.

MATERIALS AND METHODS

General

All the solvent used in this experiment were of analytical grade or GC grade. UV-Visible spectra measurements were done using Spectro (Thermo Fisher Scientific, model 4001/4) spectrophotometer Ultrospeck in methanol (λ_{max} in nm). The water was purified from water distillation plants in our laboratory. All solvents were analytical reagent grade.

Plant Material

The fresh green leaves of *Corydyline terminalis* L. Kunth were collected from the rainforest hilly area of southern part of Sabah, Malaysia. The leaves of this plant were harvested during the month of May, 2011. The leaves were collected at afternoon on May 21, 2011 and packed in polyethylene bags and bring to the laboratory and stored at 4°C until required.

Plant material grinding

The samples were washed thoroughly with fresh running water, dried under shade room temperature (25 ± 1 °C) for 3 days. The plant initially identified by morphological features and data base present in the library, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Sabah, Malaysia and a voucher specimen has been deposited at the Borneo Herbarium, Sabah, Malaysia with voucher number 031. Approximately 50 g of leaves were ground using a grinder (Blender 80115) for 20 s. The unfermented *Corydyline terminalis* L. Kunth leaves were kept in the oven at 40 °C and put in a desiccator for at least 24 h prior to analysis. The small pieces of the samples were homogenised in a grinder for 3 min to 40-mesh size. The air-dried leaves and stems of *Corydyline terminalis* L. Kunth were pulverized into powdered form.

Extraction

Coarse powders (500 g) were separately Soxhlet extracted with methanol solvent (1 L, 72 h) at the temperature between 45-65 °C. The viscous semi solid masses were dried at 40 °C in rotary evaporator frozen and lyophilized (Buchi Labortech AG, model 1, R-215). The methanol extract was (30 g) suspended in water and then defatted and finally extracted successively with hexane, chloroform, ethyl acetate and butanol to give hexane (5.03 g), ethyl acetate (4.67 g), chloroform (3.39 g), and butanol (2.58 g) and residual methanol fractions (7 g), respectively. The extract was filtered through Whatman No. 41 filter paper to obtained particle free crude extract. The residue was re extracted twice follow the same procedure and filtered. The combined extracts were concentrated and dried under vacuum.

Determination of total flavonoids

The total flavonoids content of the crude extracts of *Corydyline terminalis* L. Kunth were determined by using colorimetric method as described by Willet,[15-17] with some modifications. Methanol extracts (0.5 mL), 10% aluminium chloride (0.1 mL), 1M potassium acetate (0.1 mL) and distilled water (4.3 mL) were mixed. At room temperature the mixture samples was incubation for 30 min. The absorbance of the crude extracts was measured at 415 nm using Spectro (Thermo Fisher Scientific, model 4001/4) spectrophotometer. Quercetin was used to make the calibration curve. The calculation of total flavonoids content in the extracts was carried out in triplicate and the results were averaged.

Preliminary phytochemicals screening

One gram of hexane, ethyl acetate, chloroform, butanol and aqueous methanol crude plant extracts of the powder leaves of *Corydyline terminalis* L. Kunth were dissolved in 100 ml of its own mother solvents to obtain a stock of concentration 1% (v/v). The obtained crude extracts were subjected to preliminary biochemical screening following the methodology of Hatano[18] and Birt.[19]

SCREENING PROCEDURE

Test for alkaloids

The stock crude extract (5 ml) was added hydrochloric acid (2 ml). One milliliter of Dragendroff's reagent was added to this acidic medium. An orange or red precipitation immediately produced that indicates the presence of alkaloids.

Test for amino acids

The crude stock extract solution (1 ml) was added few drops of Ninhydrin reagent. The purple colour appearance shows the presence of amino acids.

Test for anthraquinones

The crude stock extract solution (5 ml) was hydrolysed by diluted concentrated sulphuric acid extracted with benzene. Finally dilute ammonia solution was added to it. The rose pink coloration obtained. It is suggested that the positive response for anthraquinones.

Test for flavonoids

The crude stock extract solution (1 ml) and a few drops of dilute sodium hydroxide were added. An intense yellow colour was appearance in the plant crude extract, which

become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

Test for glycosides

The crude extract was hydrolysed by hydrochloric acid for few hours on a water bath. Pyridine (1 ml) was added to the hydrolysate and a few drops of sodium nitroprusside solutions were added to it and then it was made alkaline with sodium hydroxide solution. The pink to red colour obtained shows the presence of glycosides.

Test for phytosterol

The plant crude extract solution was refluxed with solution of alcoholic potassium hydroxide till to complete saponification takes place. The whole mixture was diluted with water and then extracted with ether. The ether layer was evaporated by water bath and the residue was tested for the presence of phytosterol. The residue was dissolved with few drops of diluted acetic acid then 3 ml of acetic anhydride was added followed by few drops of Conc. H_2SO_4. The bluish green colour was appearance showed the presence of phytosterol.

Test for saponins

The crude extract stock solution (1 ml) was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm foam layer showed the presence of saponins.

Test for steroids

The crude plant extracts solution (1 ml) was dissolved in chloroform (10 ml) and added equal volume of concentrated sulphuric acid by sides of the test tube. The upper layer turns into red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for tannins

The crude extract solution (3 ml) and a few drops of 1% lead acetate were added. A yellow precipitate was formed, indicates the presence of tannins.

Test for triterpenoids

The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added following the addition of 1 ml of Conc. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Statistical analyses

Experimental results were mean ± S.D. of three parallel measurements and analyzed by SPSS 10 (SPSS Inc. Chicago, IL). Differences between means were determined using Tukey multiple comparisons and least significant difference (LSD). By using the Pearson correlation coefficient the correlation is obtained in bivariate correlations. *P* values < 0.05 were regarded significant.

Table 1: Total flavonoids content extracts of the leaves of *Corydyline terminalis*

Extract	Total flavonoids (% w/w)
Hexane extract	68.02 ± 2.15
Ethyl extract	61.50 ± 1.51
Chloroform extract	15.75 ± 0.06
Butanol extract	19.08 ± 1.92
Methanol extract	39.27 ± 1.72

The values are means ± SD of three replicates.

RESULTS AND DISCUSSION

The percentage yields of extraction of the leaves powder of *Corydyline terminalis* L. Kunth were hexane (3.19 g), ethyl acetate (16.31 g), chloroform (1.59 g), and butanol (9.50 g) and residual methanol fractions (6.34 g), respectively. The total flavonoids contents of the different organic crude plant extracts were determined by Willet method are reported as quercetin equivalents (Table 1). Among the extracts, hexane extract was containing highest (68.02%) amount of flavonoids content compounds followed by ethyl acetate (61.50%), methanol (39.27%), butanol (19.08%) and chloroform (15.75%) (Table 1). In our previous several studies, it has been reported that the yield of extractable compounds was highest in methanol extract from the leaves, peel and seeds of pomegranate in comparison with the solvents such as chloroform, butanol, ethyl acetate and hexane. Furthermore, the extraction of flavonoids contents from the leaves, peel and seeds is commonly achieved with methanol or aqueous ethanol.

The result obtained in the present investigation (Table 2), the ethyl acetate, chloroform, butanol, aqueous ethanol and methanol extracts of the leaves powder of *Corydyline terminalis* L. Kunth showed the presence of alkaloids, amino acids, flavonoids, glycosides, saponins, steroids, tannins and triterpenoids. Further, all the organic extracts of the leaves showed the absence of anthraquinones. In the hexane extract, all the group alkaloids, amino acids, flavonoids, glycosides, saponins and triterpenoids was absence except triterpenoids and steroids. .

All the herbs and herbal extracts contain different chemical constituents with biological activity that can be show of valuable therapeutic and medicinal index. Most of the protective effect of medicinal plants, fruits and vegetables has been attributed by active biochemicals, which are the non-nutrient plant compounds. Different type's active biochemicals have been found to possess a wide range of activities, which may help in protection against uncureable

Table 2: The analysis of biochemical in the hexane, ethyl acetate, chloroform, butanol and methanol crude extract of *Corydyline terminalis*

Biochemicals	Inference				
	Hexane	Ethyl acetate	Chloroform	Butanol	Methanol
Alkaloids	+	+	+	+	+
Amino acids	–	+	–	–	+
Anthraquinones	–	–	–	–	–
Flavonoids	+	+	+	+	+
Glycosides	–	+	–	+	+
Phytosterol	–	–	–	–	+
Saponins	–	+	+	+	+
Tannins	–	+	+	+	+
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+

+ = presence; - = absence

diseases. Biochemicals and phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have antiinflammatory effects.[20-24] Some complex glycosides, flavonoids, tannins and alkaloids have also very high hypoglycemic activities.[25-26] Recently by Allan[4] have reported that saponins possess hypocholesterolemic and antidiabetic properties. The chemical constituents such as mono di and triterpenoids have also been shown to decrease or reduce the blood sugar level in animal studies.[27-30] Almost all high molecular weight such as glycosides, steroids and triterpenoids showed the analgesic properties.[31-35] The steroids and saponins are also responsible for central nervous system activities.[9]

The biochemical and phytochemicals screening of the hexane, ethyl acetate, chloroform, butanol, aqueous ethanol and methanol extracts of *Corydyline terminalis* L. Kunth powder leaves used in this present study revealed that the crude extracts contained alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids (Table 2).

This study is only a preliminary study of the occurrence of certain properties of *Corydyline terminalis* L. Kunth leaves an in-depth study will provide a good concrete base of all the biochemicals and phytochemicals functions mention above.

CONCLUSION

In this present study, we have found that biologically active biochemical and phytochemicals were present in the ethyl acetate, chloroform, butanol and methanol extracts of *Corydyline terminalis* L. Kunth. The high flavonoids contents of *Corydyline terminalis* L. Kunth leaves crude extracts may be due to the presence of bioactive phytochemicals. Further studies are in progress in our laboratory is to isolate the bioactive components from the leaves of *Corydyline terminalis* L. Kunth.

ACKNOWLEDGEMENTS

The authors are grateful to Associate Prof. Dr. Vijay Kumar, Acting Director, Biotechnology Research Institute, University Malaysia Sabah, Malaysia for their continuous encouragement during the work and the use of all laboratory facilities. We are also thanks to Biotechnology Research Institute, University Malaysia Sabah, Malaysia for providing the seed money. Thanks are also due to Mr. Emran Raga, Laboratory Assistant, Biotechnology Research Institute, University Malaysia Sabah, Malaysia for his help to assist our entire work.

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Pharmacognostic Standardization of the Leaves and Root Bark of *Caesalpinia benthamiana*

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ABSTRACT

Caesalpinia benthamiana (Baill.) Herend. and Zarucchi (*Mezoneuron benthamianum* Baill.) (Caesalpiniaceae) has been traditionally used in management of erectile dysfunction, dysentery, urethral discharges, skin diseases and wounds. Despite a long tradition of use in the treatment of various ailments, no systematic pharmacognostic standardization work has been carried out on *C. benthamiana*. One major obstacle in the systematic exploration of the plant may be the non-availability of authentic plant material. In the present investigation, various pharmacognostic standards for the plant have been generated including the macro and micro morphological studies, one of the WHO accepted parameters for identification of medicinal plants by way of establishing the salient diagnostic characters and constants. This was carried out on the leaves and root bark of *C. benthamiana.*

The leaves were bipinnate, oblong, with entire margin. The apex was obtuse, and possessed a symmetric base and reticulates venation. The terminal leaves were however obovate in shape. Actinocytic and paracytic types of stomata were observed. Trichomes were uniseriate and unicellular and epidermal cells were found to be wavy. Surface data analysis revealed the stomatal index to be 1.69% to 11.11% for the upper surface and 16.94% to 28.52% for the lower surface. Vein-islet number was recorded as 12.5 to 16.5 whilst the veinlet termination number was 22.25 to 35 with palisade ratio ranging between 11.25 and 13.75. The water- soluble extractive value was 9.2% and 3.7% for the leaves and root bark respectively. Whilst the alcohol-soluble extractive value for the leaves was found to be 6.7% and 2.6% for the root bark. The total ash value determinations were observed to be 5.6% for the leaves and 7.9% for the root bark. The result of this study may be useful in setting diagnostic indices for the identification and preparation of a monograph for the plant.

Key words: *Caesalpinia benthamiana*, Pharmacognostic studies, Quantitative microscopy and Extractive values

INTRODUCTION

Caesalpinia benthamiana (Baill.) Herend. and Zarucchi (*Mezoneuron benthamianum* Baill.) (Caesalpiniaceae),[1] is an African tropical shrub found mostly in the secondary forest zones.

The roots of *C. benthamiana* are considered to be an effective dysentery remedy in Ghana.[2] The powdered roots are used mixed with shea butter or palm kernel oil to treat skin diseases and wounds in Ghana.[3] A decoction of the root, bark, and leaves is used in Guinea for urethral discharges.[2]

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DOI: 10.5530/pj.2011.24.6

An infusion of the dried root is drunk or used as a bath for general malaise in Senegal.[4] The aqueous decoction of the roots is used in traditional medicine as aphrodisiacs and the vasorelaxant properties have been reported.[5]

The anti-diarrhoeal effects of the aqueous extract of the plant have also been studied. The results obtained showed that the plant possessed anti-diarrhoeal activity due to its inhibitory effects on gastrointestinal propulsion and intestinal fluid accumulation.[6] The analgesic, antipyretic and anti-inflammatory effects of the aqueous extract of the plant have been also been evaluated in mice, rats and rabbits. The data obtained show that *C. benthamiana* root bark extract possesses analgesic and antipyretic activities but lacked anti-inflammatory properties.[6] In a phytochemical investigation conducted by Dickson *et al.*,[7] the bioactivity-guided fractionation of the light petroleum extract of the root bark of the plant led to the isolation of two novel cassane diterpenoids, designated as benthaminin 1 and 2. A third compound, a deoxy form of caesaldekarin C (also

referred to as methyl vouacapenate) which had previously been isolated from *Caesalpinia major*, *Caesalpinia bonducella, Vouacapoua americana and Vouacapoua macropetala*, was also isolated, together with beta-sitosterol and stigmastenone. The antibacterial and antioxidant activities of these cassane diterpenoids have also been assessed.[7] The resistance modifying activities of extracts from this plant on standard antibiotics against *Staphylococcus aureus* have also been assessed by the same group of researchers. A 4-fold potentiation of the activity of norfloxacin was observed for the ethanol extract, whilst the petroleum spirit extract resulted in a 2-fold potentiation.[8]

Despite a long tradition of use in the treatment of various ailments, *C. benthamiana* has not been explored properly by way of establishment of standards in the identification and quality control of this plant. The cassane-type diterpenoids possessing antimicrobial, antioxidant and wound healing properties isolated from the plant could serve as leads in the search for new biomolecules as drugs. The need to standardize this plant can therefore not be overemphasized. It is also worthwhile to note that some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been prepared appropriately and standardized. The use of *Artemisia annua*, *Digitalis* and *Senna* leaves are a few examples. The establishment of the pharmacognostic profile of the leaves and root bark of *C. benthamiana* will assist in standardization, which can guarantee quality, purity and identification of samples to ensure that only the authentic plant is explored properly for its traditional claims.

MATERIALS AND METHODS

The fresh leaves and roots of the plant was collected from the Ayeduase in the Ashanti Region of Ghana and authenticated by Mr. Henry Sam of the Department of Herbal Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, where voucher specimens were deposited with the numbers RADCB 01 and RADCB11 respectively for the leaves and roots of the plant.

Organoleptic evaluation

In the organoleptic evaluation, various sensory parameters of the plant material such as colour, odour, taste and texture were investigated.[9]

Macroscopic evaluation

The following macroscopic characters for the fresh leaves were noted: the type of leaves, its arrangement, colour, shape, length and width of the leaves, size and surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture.[9,10]

Microscopic evaluation

Leaves were cut into smaller sizes and cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence /absence of the following were observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse section through the fresh petiole of the leaf was also examined.

Preliminary phytochemical investigation

The leaf and root bark powders of the plant were separately subjected to Soxhlet extraction using 70% ethanol. Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins (Ferric chloride test), cardiac glycosides (Keller-Killiani and Kedde tests), alkaloids (Mayer's; Dragendorff's; Wagner's and 1% picric acid reagents), Saponin glycosides (frothing and haemolysis tests), anthracene derivatives (Borntrager's test for combined and free Anthraquinones) and Cyanogenetic glycosides (sodium picrate paper test).[10,11,12,13]

Quantitative investigations

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein- islet number and veinlet termination number were carried out on cleared sections of the leave. Other parameters determined for the powdered leaves were moisture content, total ash, acid - insoluble ash, water - soluble ash, alcohol (90% ethanol) and water soluble extractive values.[14] The moisture content of the powdered leaves and root bark of the plant was also determined using the Dean-Stark apparatus.[9]

Determination of total ash

2 g of the powdered leaves and root bark of *C. benthamiana* were weighed separately in a pre-weighed ash-less filter paper and incinerated at 400°C for about 3-4 min or until the vapours completely ceased. The temperature was gradually reduced to come to normal and then the ash was collected and weighed.

Determination of alcohol soluble extractive

Accurately weighed powder (10 g) of both leaves and root bark were taken separately and macerated with 100 ml of 95% alcohol for 24 hours. The contents were frequently shaken during the first 6 hours and allowed to remain for another 18 hours. After 24 hours, the extract was filtered and 20 ml of the filtrate was evaporated to dryness. The extract was dried at 105°C to a constant weight.

Determination of water soluble extractive

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration in this instance.

RESULTS

Macroscopically, the plant possess compound leaves which are bipinnate, alternate in arrangement, apex and base are obtuse, margin is entire, venation is reticulate, shape oblong with terminal leaves being obovate. Full length of leaves 33-38 cm, Six (6) pairs of pinnae, with length of each pinnae being 6.5-7.5 cm and four (4) pairs of leaflets on each pinnae. The average size of individual leaf is 2.0-5.0 cm ± 0.5 (length) and 1.5-3 cm ± 0.3 (width). Fresh leaves are green in colour, odourless with a gritty texture. It is petiolated with the length of petiole between 4-5 cm, shape of petiole cylindrical and brownish-green in colour.

The leave arrangement may be similar to that of *C. spinosa* but whereas the leaves of *C. spinosa* lack petioles those of *C. benthamiana* are petiolated.[15]

The length of the leaves of *C. benthamiana* falls between 33 to 38 cm which is within those of *C. pulcherrima* which are also between 20 cm and 40 cm but are longer than those of *C. gilliesii* which falls between 10 cm and 15 cm long.[16]

The leaves of *C. benthamiana* have two types of stomatal arrangements and these are the actinocytic and paracytic types. The types of trichomes observed were uniseriate clothing hairs and unicellular hairs. The epidermal cells were found to be wavy.

Micromorphological features revealed that anticlinal walls are thin and wavy. The type of stomata revealed actinocytic arrangement and these were few as compared to the paracytic ones which were more. Uniseriate covering trichomes are present on both surfaces. It has Isobilateral leaf arrangement. The midrib bundle is surrounded by a zone of pericyclic fibres possessing double layered parenchymatous cells. The roots are brown in colour and the texture is gritty. Vein islet number was determined to be 12.5 to 16.5, veinlet termination number ranging from 22.5 to 35, stomatal index of 1.69% to 11.11% for the upper surface and 16.94% to 28.52% for the lower surface and palisade ratio of 11.25 to 13.75. Water- soluble extractive values were 9.2% and 3.7% for the leaves and roots respectively. The alcohol-soluble extractive value of 6.7% was obtained for the leaves and 2.6% for the roots. Also, the total ash values for both leaves and roots were 5.6% and 7.9%. Phytochemical evaluation revealed the presence of true tannins, pseudotannins and terpenoids mainly for both leaves and roots. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for the various actions of *C. benthamiana*. See Tables 1 to 6 numerical and quantitative values as well as morphological descriptions.

DISCUSSION

The standardization of a crude drug is an integral part of establishing its correct identity.[17] *Caesalpinia benthamiana* is employed in ethnomedicine in the management of various disease states without standardization. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs.[18] The vein islet, and vein termination numbers and the other parameters determined in the quantitative microscopy, are relatively constant for plants and can be used to differentiate between closely related species.[19] Also, the physical constant evaluation of the crude drugs is an important parameter which is a valuable tool in detecting adulteration or improper handling of crude drugs. The moisture content of the powered drugs may be said to be high since the BP stipulates an allowable value of not more than 10%. Any value beyond this encourages microbial growth and subsequent deterioration of the stored powdered drugs.

Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly significant in the evaluation of purity of the crude drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.[20] The macro - and micro – morphological features of the leaf described, distinguishes it from other members of the genera. For example whereas the leaves of *Caesalpinia spinosa* lack petioles those of *Caesalpinia benthamiana* are petiolated.[15] The length of the leaves of *Caesalpinia benthamiana* falls between 33 to 38 cm which is within the range for those of *Caesalpinia pulcherrima* which are also between 20 cm and 40 cm but the leaves of *Caesalpinia gilliesii* are shorter, falling within the range of 10 cm and 15 cm.[16]

By and large, the pharmacognostic constants including extractive values, ash values and the phytochemical profile of *Caesalpinea benthamiana* obtained for the leaves and root bark of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for the proper identification and use of this plant.

CONCLUSION

These parameters which are being reported for the first time, could serve as useful information in preparing a monograph of the plant which can be locally incorporated into the Ghana Herbal Pharmacopoeia which may ultimately serve as a significant addition to international herbal pharmacopoeias. Any crude drug which is claimed to be

Caesalpinea benthamiana but whose characters significantly deviate from the accepted standards above may be considered to be contaminated, adulterated, substandard or fake.

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Pharmacognostic, Physico-chemical and Phytochemical Evaluation of Leaves of a Species of 'Paarshva-pipla': *Ficus arnottiana*

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ABSTRACT

Introduction: 'Paarshva-pipla' is an important drug mentioned in the traditional medicinal texts. However, two completely different species are used as 'Paarshva-pipla': *Thespesia populnea* (Malvaceae) and *Ficus arnottiana* (Moraceae). Recent pharmacological findings indicate that leaves of *Ficus arnottiana* possess significant anti-ulcer activity which complies with the claims made in the traditional medicinal texts regarding 'Paarshva-pipla'. However, no conclusive pharmacognostic study of the leaves has been performed yet. **Methods:** The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation. **Results:** Main characters of the transverse section include presence of bi-layered palisade, cystoliths, pericyclic fibers and perimedullary phloem. Diagnostic characters of powder include pericyclic fibers, cystoliths, anomocytic stomata, horse-shoe shaped xylem vessels and xylem vessels with annular thickening. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, cardiac glycosides, saponins, flavonoids, phenolics and carbohydrates. **Conclusion:** Such a study would help in distinguishing *Ficus arnottiana* from *Thespesia populnea* and make way for isolation of phytoconstituents, therapeutic investigations and standardization of formulations containing its leaf material. Most importantly, it may throw light on the true botanical identity of 'Paarshva-pipla'.

Key words: *Ficus arnottiana,* Indian Rock fig, Moraceae, Paarshva-pipla, *Urostigma arnottiana*

INTRODUCTION

Ficus arnottiana Miq. syn. *Urostigma arnottiana* (Family - Moraceae) is also known as (English) Indian rock fig, (Hindi) Paras Pipal and (Sanskrit) Paarshva-pipla, Plaksha.[1] Its leaves are used in stomach ulcers.[2] The present investigation deals with the qualitative and quantitative microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation.

MATERIALS AND METHODS

Collection and authentication of leaves

Leaves of *Ficus arnottiana* were collected from the garden of Primary Health Center, Gadhaka near Rajkot, Gujarat, in July 2010. Herbariums and voucher sample were prepared and deposited in Department of Pharmacognosy, R. K. College of Pharmacy (Voucher no. RKCP/COG/06/2010). Authentication was done by Dr. A. N. Pandey, Department of Biosciences, Saurashtra University.

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DOI: 10.5530/pj.2011.24.7

Pharmacognostic studies

Morphology of fresh leaves of *Ficus arnottiana* was studied. Photomicrography of stained and unstained transverse sections of fresh leaves was performed using USB camera and software. Leaf constants were established using camera lucida. The leaves were dried under shade, powdered to 60#, stored in airtight containers and used for powder study and quantitative microscopy (Table 1).[3]

Physico-chemical evaluation

Various physico-chemical parameters like loss on drying, ash values (total ash, water soluble ash and acid-insoluble ash) and extractive values (water soluble, alcohol soluble and petroleum ether soluble extractives) were established using the powdered drug (Table 2).[4]

Phytochemical study

The powder was extracted separately with 500 ml each of distilled water and alcohol at 70 °C for two hours each. Various phytoconstituents present in the leaves were detected by their respective chemical tests using the appropriate extracts (Table 3).[5-12]

RESULTS AND DISCUSSION

Pharmacognostic study

Macroscopical characteristics

Leaves are simple, 7.5-15 cm × 3.2-6.3 cm, ovate-oblong or elliptic-lanceolate, apex acuminate, margin wavy, surface glabrous and glaucous, texture membranous, base symmetric, venation reticulate. Color of upper surface is dark green and lower surface is light green. Three prominent veins seem to be arising from the base of the lamina. Petiole is 8-10 cm long, angular and reddish-brown (Figure 1).

Table 1: Quantitative microscopy

Leaf constant	Mean value ± SD
Stomatal Index	
Upper surface	4.9 ± 0.4
Lower surface	15.9 ± 0.5
Palisade ratio	5 ± 1
Vein islet number	12 ± 1
Vein termination number	15 ± 1

Number of observations = 10
SD = Standard Deviation

Table 2: Physicochemical evaluation

Parameter	Mean ± SD
Loss on drying	11.5 ± 0.3%w/w
Ash values	
Total ash	13.1 ± 0.3%w/w
Acid insoluble ash	0.9 ± 0.1%w/w
Water soluble ash	3.8 ± 0.3%w/w
Extractive values	
Water soluble extractive	18.4 ± 0.3%w/w
Alcohol soluble extractive	6.3 ± 0.2%w/w

Number of observations = 3
SD = Standard Deviation

Table 3: Phytochemical screening

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	+ve
	Hager's test	+ve
	Wagner's test	+ve
	Mayer's test	+ve
Flavonoids	Shinoda test	+ve
	Lead acetate test	+ve
Phenolics	Ferric chloride test	+ve
	Folin ciocalteu test	+ve
Sterols	Salkowski test	+ve
and triterpenoids	Libermann-Buchardt test	+ve
Cardiac glycosides	Legal test	+ve
	Baljet test	+ve
	Keller Killiani test	+ve
Saponins	Foam test	+ve
	Lead acetate test	+ve
Anthraquinone	Borntrager test	–ve
glycosides	Modified Borntrager test	–ve
Carbohydrates	Fehling's test	+ve
	Molisch test	+ve
Gums	Ruthenium red test	+ve

Figure 1: Leaves of *Ficus arnottiana* *(A- Upper surface, B-Lower surface)*

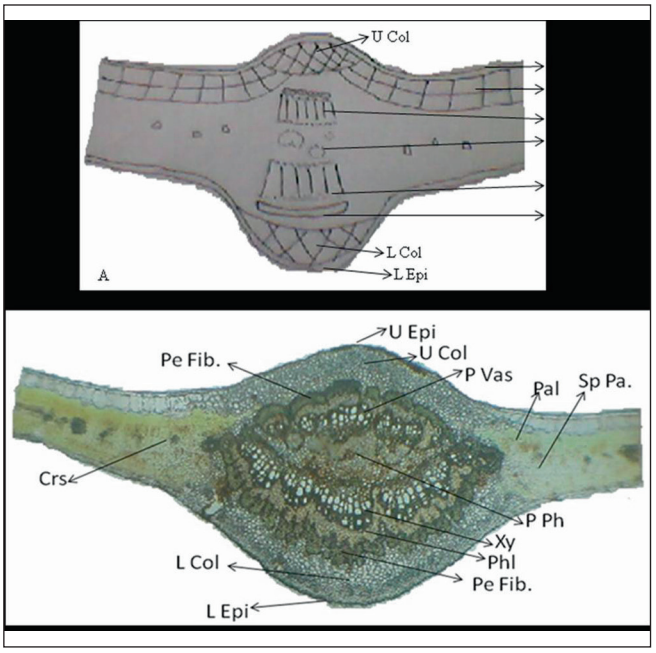

Figure 2: A – Schematic diagram (T.S.), B- Detailed T. S. of leaf *(U Co, Upper Collenchyma; U Ep, Upper Epidermis; L Co, Lower Collenchyma; L Epi, Lower Epidermis; Pal, Palisade; Xyl, Xylem; Phl, Phloem; Pe Fib, Pericyclic fiber; Sp Pa, Spongy parenchyma; PPh, Perimedullary phloem; Cys, Cystolith; Va Bun, Vascular bundle)*

Microscopy: Transverse section

Lamina of the transverse section shows large, tabular epidermal cells covered by thick cuticle. Underlying the upper epidermis are bi-layered, compact, radially elongated palisade cells followed by spongy mesophyll composed of 3-4 layers of loosely arranged parenchymatous cells containing calcium carbonate crystals which dissolve in acetic acid (cysoliths) and a cavity at the lower epidermis. A well-developed collenchyma is seen beneath each epidermis in the mid-rib. Ground tissue of the mid-rib consists of loosely arranged polygonal parenchymatous cells having calcium oxalate prism crystals and orange color pigment. Vascular bundles are bicollateral, crescent shaped; with patches of perimedullary phloem and presence of secondary vascular bundles. A continuous layer of pericyclic fibers is present surrounding vascular bundles. Trichomes are rare. (Figure 2, 3)

Microscopy: Powder characteristics

It is dark green with no distinct odor or taste. The important diagnostic features of the powder include parts of epidermis in surface view showing straight walled epidermal cells and anomocytic stomata, xylem vessels with annular thickening, fragments of horse-shoe shaped xylem vessels, cystoliths, orange color matter and bundles of pericyclic fibers. (Figure 4)

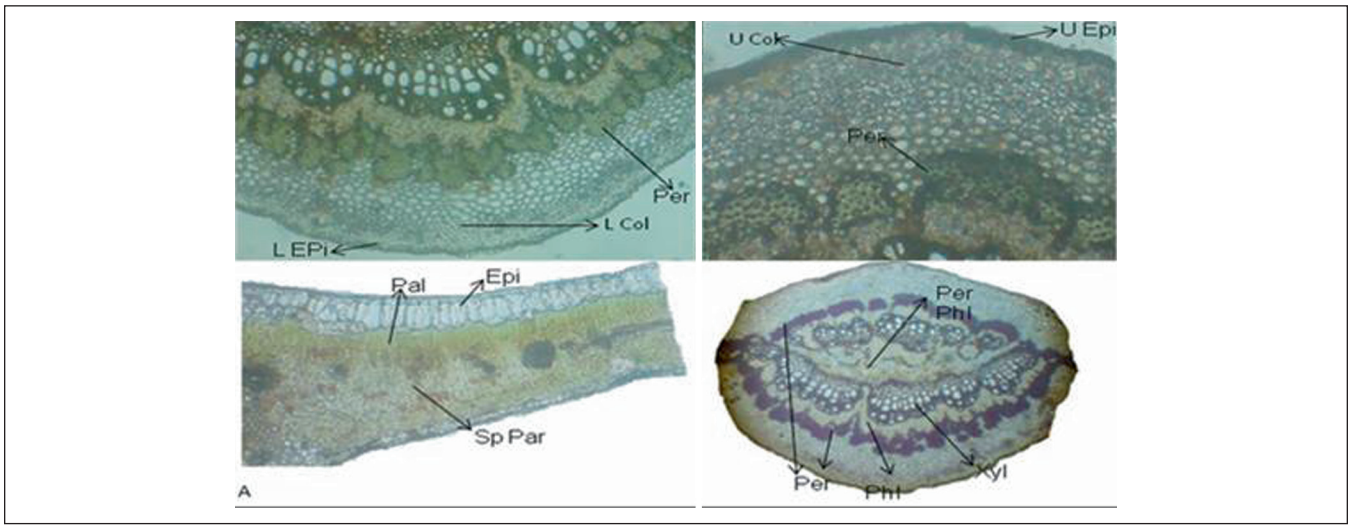

Figure 3: T. S. of leaf of *Ficus arnottiana* showing single enlarged portions (X400)
(U Co,Upper Collenchyma; U Ep, Upper Epidermis; L Co, Lower Collenchyma; L Epi, Lower Epidermis; Pal, Palisade; Xyl, Xylem; Phl, Phloem; Pe Fib, Pericyclic fiber; Sp Pa, Spongy parenchyma; PPh, Perimedullary phloem)

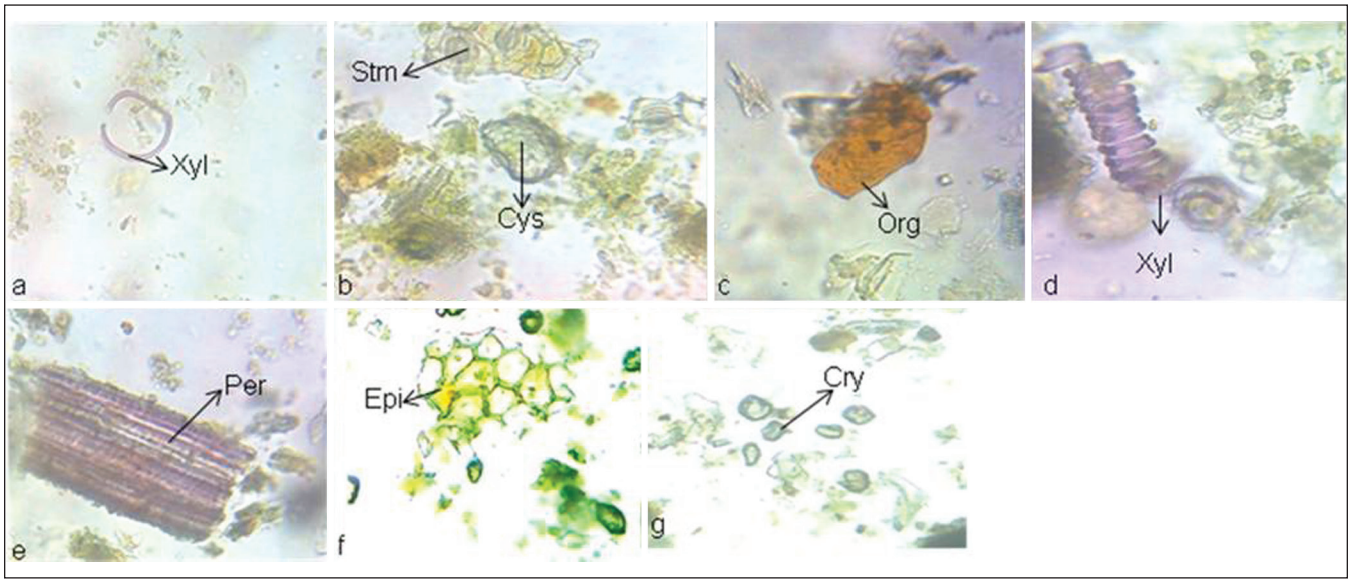

Figure 4: Powder study (X400)
(a, Horse-shoe shaped xylem vessel; b, Cystoliths and anomocytic stomata in surface view; c, Orange matter; d, Xylem vessels with Annular thickening; e, Bundle of pericyclic fibers ; f, Epidermis in surface view; g, Calcium oxalate prism crystals.)

CONCLUSION

The present work deals with the macroscopic, microscopic, physicochemical and phytochemical evaluation of the leaves of *Ficus arnottiana*. Diagnostic microscopic characters include pericyclic fibers, cystoliths, anomocytic stomata, horse-shoe shaped xylem vessels and xylem vessels with annular thickening. Various physicochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, cardiac glycosides, saponins, flavonoids, phenolics, carbohydrates, sterols and triterpenoids. This indicates that these both the plant can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds. Development of such a monograph would help in distinguishing *Ficus arnottiana* from *Thespesia populnea,* thereby providing evidence on the true botanical identity of 'Paarshva-pipla' and paving the way for isolation of phytoconstituents, therapeutic investigations and standardization of formulations containing its leaf material.

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Pharmacognostical Evaluation of Different Parts of *Coleus amboinicus* lour., Lamiaceae

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ABSTRACT

Coleus amboinicus Lour. belonging to the family Lamiaceae commonly known as Karpuravalli, Omavalli in Tamil, Patta ajavayin, Patharcur in Hindi, Country borage in English is a large succulent aromatic perennial herb. The leaves of this plant have been used in malarial fever, hepatopathy, renal and vesicle calculi, cough, chronic asthma, hiccough, bronchitis, anthelmintic, colic and convulsions. This paper deals with the pharmacognostical evaluation of leaves, stems and roots of *Coleus amboinicus* by using different parameters which include morpho-anatomical studies, physicochemical properties and fluorescence analysis to set the quality control parameters for the raw material.

Key words: quality control, microscopy, Physico-chemical, Morpho-anatomical

INTRODUCTION

The plant *Coleus amboinicus (synonym: Plectranthus amboinicus, Coleus aromaticus)* commonly known as Country borage, Indian borage, is a dicotyledonous plant belonging to the family *Lamiacea*.[1,2] It is a large succulent aromatic perennial herb. Much branched, fleshy highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed through out India, cultivated in the gardens. It is a folkloric medicinal plant used to treat malarial fever, hepatopathy,[3] renal and vesical calculi, cough, chronic asthma,[4] hiccough, bronchitis, helminthiasis, colic, convulsions and epilepsy.[5] It is used to treat colds and cough as well as arthritic inflammations.[6] Its insect repellent properties have been tested[7] and another member of the Coleus genus, *C. aromaticus*, has been found to cause reduction in egg laying capacity, retard in adult emergence and weight loss in the pulse beetle *Callosobruchus maculatus*.[8] A moderate allelopathic effect of the powdered leaves of *C.amboinicus* against the water hyacinth is also on record.[9] Studies performed in India demonstrated the "fungistatic" properties of the essential oil of this plant.[10] The Phytochemical study reveals the presence of various flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin and volatile oils in the leaves.[11] Ethanolic and aqueous leaf extracts of the plant has been found possess significant diuretic activity.[12] The plant is also known to contain the constituents responsible for cytotoxicity and anti bacterial activity.[13]

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DOI: 10.5530/pj.2011.24.8

METERIALS AND METHODS

Plant material

Coleus amboinicus was collected from the surrounding Shimoga city, Karnataka and authenticated by Prof. D. Rudrappa, Dept. of Botany, S.R.N.M. College, Shimoga. The collected plants are washed thoroughly with water, all the three parts i.e. leaves, stems and roots were separated and dried in shade at room temperature and powdered using hand mill to make a coarse powder. Then they are stored in well-closed light resistant container until further use.

Morpho-Anatomical studies of *Coleus amboinicus*

The macroscopy and microscopy of the plant was studied according to the methods of Brain & Turner,[14] the cross sections were prepared and stained.

Physico-chemical analysis

Air dried plant material was used for the quantitative determination of ash and extractive values were determined as per the WHO guidelines.[15] Fluorescence analysis of the extract(s) was carried out by the method of Chase & Pratt.[16]

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedure described by Kokate et al.[17] and Harbone.[18] Total phenol content of the extracts was determined by using the Folin-Ciocalteu method.[19] Total flavonoid content was determined as described by Singleton & Rossi.[20] The amount of total alkaloids and total saponins was determined according to method given by Rajpal.[21, 22]

Thin Layer Chromatography

All the three extracts were subjected for thin layer chromatography. Various mobile phases were tried and the one with maximum number of spots was selected. The most suitable mobile phase was found to be Toluene: Dioxan: Glacial acetic acid (90:25:4). All the three plant extracts were dissolved in methanol and applied to pre-coated TLC silica gel plates (silica gel 60 F_{254}, AluGram, Germany). Chromatograms were developed and were examined under UV and daylight as well as after spraying with Anisaldehyde-sulphuric acid reagent to detect the presence of different phytoconstituents.

RESULTS AND DISCUSSIONS

Macroscopic Characters

Leaf, stem and root parts of *Coleus amboinicus* were evaluated on morphological parameters. Leaves are fleshy, thick, acute apex, pinnate, dentate margin and symmetrical base. Stems are flexible, smooth, small, and fleshy and having fibrous structured with large number of nodes and inter-nodes (Figure 1A). Roots are cylindrical to torus and sharp edges at the base with large numbers of rootlets. The details are given in Table-1.

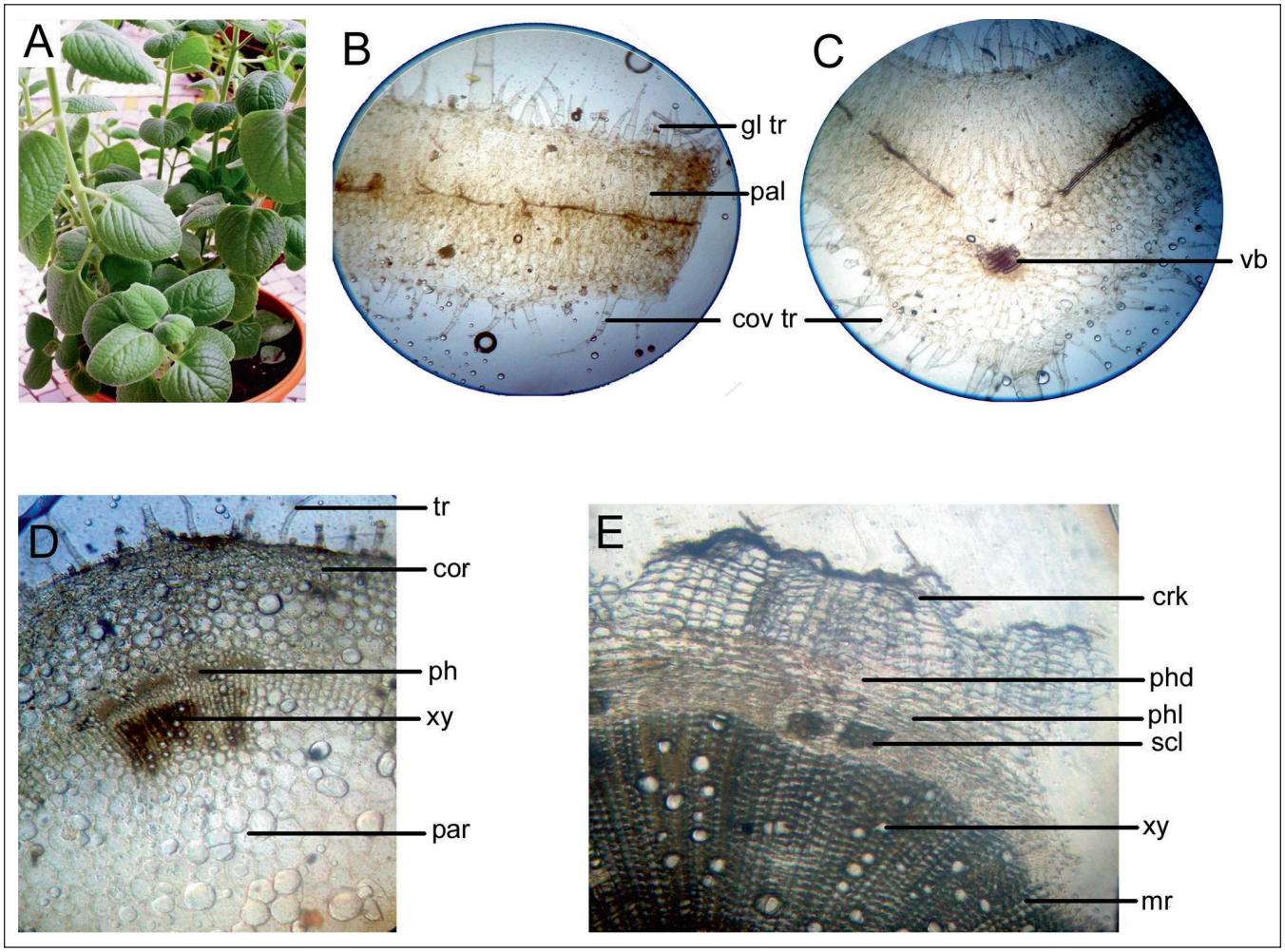

Figure 1: Morpho-anatomical features of *Coleus amboinicus*
A. Photograph from the natural habitat showing leaves and stem, B. Transverse section of lamina of the leaf, C. Transverse section of midrib of the leaf, D. Transverse section of stem, E. Transverse section of root. gl tr, glandular trichome; pal, palisade cells; cov tr, covering trichome; vb, vascular bundle; tr, trichome; cor, cortex; ph, phloem; xy, xylem; par, parenchyma; crk, cork; phd, phelloderm; phl, phellogen; scl, sclerenchyma; mr, medullary rays

Table 1: Organoleptic evaluation of leaves, stem and root parts

Parameters	Leaf	Stem	Root
Colour	Light green	Green to pink	Brown
Odour	Highly aromatic	Aromatic	Aromatic
Taste	Slightly bitter to acrid	Slightly bitter to acrid	Aromatic
Size	Max. 6.5 cm in length, and max. 6 cm in width	70 to 80 cm in length	Extended up to 15 to 20 cm

Microscopic Characters of leaf

The transverse section of leaf shows the dorsiventral characters. The important tissues in the lamina and midrib regions are as follows-

Upper epidermis is made up of single-layered rectangular cells with cuticle. Both covering and glandular trichomes are seen. Covering trichomes are uniseriate, multicellular with acute apex. The basal cells of the trichomes are comparatively wider. Covering trichomes are made up of 2-3 cells. Glandular trichomes made up of 2-3 celled base and unicellular head (Figure 1B).

Mesophylls is differentiated into 2 regions, towards upper epidermis single-layered compact and radically elongated palisade cells and towards lower epidermis more or less spherical parenchymatous cells without intercellular space containing chloroplast. Lower epidermis is identical to upper epidermis and is discontinuous due to the presence of Stomata and numerous covering and glandular trichomes.

Midrib appears as plano-convex with flat dorsal side and hemispherical ventral side. In the epidermal layer, lamina is continuous with the midrib region. Vascular bundles are small, single and less prominent and consist of 4-6 rows of xylem and thin arc of phloem (Figure 1C). Surface preparation shows diacytic type of stomata.

Microscopic Characters of Stem

The transverse section of stem is almost circular in nature. Margin is silky due to presence of numerous multicellular covering trichomes. Some of the important tissues from the periphery to the centre as follows-

Epidermis contains single row of flattened closely arranged cells with cuticles. Some of the cells of epidermis developed into multicellular covering trichomes. Base is 2-3 celled, uniceriate and head is unicellular in nature.

3 distinct portions can be clearly observed in the cortex region. The outer most 3-4 layers of cholenchymatous cells are closely arranged where the cells are thickened due to deposition of pectin. It is followed by 4-5 layers of loosely arranged parenchymatous cells with inter cellular space. The cells are more or less spherical in nature. The inner most layer of the cortex (Endodermis) is made up of closely arranged radially elongated parenchymatous cells. The endodermis separates the cortex region from the stellar region.

Around 7-9 collateral, conjoint, opened type of vascular bundle are arranged in ring. Phloem is towards the periphery and containing Sieve tubes, companion cells, phloem-parenchyma, and phloem fibers. Cambium is made up of thin-walled radially arranged cells.

Xylem is made up of meta-xylem, proto-xylem and contains xylem-parenchyma and xylem fibers. Below the endodermis, patches of pericyclic fibers are present in the form of semi-lunar shape which forms the Bundle-cap. Pith region is large and made up of thin-walled polygonal parenchymatous cells with inter-cellular spaces (Figure 1D).

Microscopic Characters of Root

The transverse section of the matured root presents a circular outline with following important tissues- from periphery to the centre.

Cork is stratified and consists of smaller, suberised unlignified cells up to 5-6 rows and larger lignified cells up to 6-8 rows in radial depth. Phellogen is distinct and seen as thin-walled cells. Phelloderm is 6-8 layered immediately below the Phellem. Cells are parenchymatous and with inter-cellular spaces.

Secondary phloem lies towards outside, consists of sea-tubes, companion cells, and phloem parenchyma. Secondary xylem lies towards inside and which forms main bulk of the root. It is made up of meta-xylem and proto-xylem arranged alternatively in a row. Xylem consists of vessels, wood-fibers and lignified parenchyma. Medullary rays runs radially from centre to the cortex through phloem. Medullary rays are lignified in nature in 2-3 cells wide (Figure 1E).

Determination of physicochemical parameters:

Various physicochemical parameters were evaluated for the leaf, stem and root parts as per WHO guidelines. Significant amount of acid insoluble ash had been detected which indicates presence of various silicacious substances. Cellulosic substances also contributed significantly in total ash as indicated by water soluble ash.

The plant contains fewer amounts of non-polar substances in comparison to polar substances, as extractive value

increases with the increase in the polarity of solvents. However, alcoholic and aqueous extractives showed significant yields. Significant amount of moisture had been also found in air-dried materials (Table-2).

Preliminary phytochemical screening

The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, saponins in all the three extracts and carotenoids were detected in leaf and stem extracts. The total polyphenols, total flavanoids, total alkaloids and total saponis in the different parts of the plant are given in the table 3. The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Thus the fluorescence analysis of the drug powder was carried out and data is presented in the Table 4, 5 and 6.

Thin Layer Chromatography

The solvent system which showed maximum separation of constituents for all the extracts was- Toluene: Dioxan: Glacial acetic acid (90:25:4). In visible light and in UV both short and long wavelengths, the Rf value of quercetin matches with the Rf values of the first spots of all the three extracts. This indicates the presence of quercetin in all the three parts of the plant. In visible light and in UV both short and long wavelengths leaf extract showed presence of about 10 components, whereas stem and root extract showed less components. But when sprayed with anisaldehyde-sulphuric acid reagent, about 14 spots have been detected with both leaf and stem extracts, whereas about 16 spots detected with root extract. This indicates presence of more numbers of triterpenoids and saponins in the root extract (Figure 2).

Table 2: Physicochemical parameters of leaves stem and root parts

Parameters	Leaf	Stem	Root
Extractive Values			
Water soluble extractive value	30.38 ± 0.30%	14.93 ± 0.16%	11.13 ± 0.21%
Alcohol soluble extractive value	13.10 ± 0.33%	7.00 ± 0.20%	4.40 ± 0.17%
Ether soluble extractive value	3.50 ± 0.19%	1.08 ± 0.09%	0.88 ± 0.11%
Ash Values			
Total ash	21.03 ± 0.03%	11.43 ± 0.02%	9.23 ± 0.02%
Water soluble ash value	7.90 ± 0.12%	5.85 ± 0.22%	8.05 ± 0.31%
Acid insoluble ash value	2.05 ± 0.21%	0.95 ± 0.18%	1.75 ± 0.13%
Loss On Drying	11.96 ± 0.15%	9.30 ± 0.10%	8.29 ± 0.51%

* Values are in mean± Standard deviation, where as n=6.

Table 3: Amount of various active constituents in different part of *Coleus amboinicus*

Extracts	Amount of major active constituents (% w/w)			
	Total Phenolics	Flavonoids	Alkaloids	Saponins
CALE	19.62 ± 0.83	4.21 ± 0.39	4.3 ± 0.74	2.09 ± 0.33
CASE	14.30 ± 0.49	3.59 ± 0.41	1.28 ± 0.50	0.84 ± 0.15
CARE	12.91 ± 0.60	1.54 ± 0.25	0.60 ± 0.19	0.52 ± 0.16

Values represent the mean ± SD of five readings
CALE - *Coleus amboinicus* leaf extract, CASE - *Coleus amboinicus* stem extract, CARE - *Coleus amboinicus* root extract

Table 4: Fluorescence characters of powdered Leaf

Particulars of treatment	Under Visible light	Under UV light	
		Short Wavelength	Long Wavelength
Water	Pale brown	Light-black	Brownish-black
5% Picric acid	Pale brown	Black	Brownish-black
NH_3 solution	Greenish brown	Black	Greenish-black
Methanol	Brown	Black	Brownish-black
Acetic acid	Purple	Light bluish-black	Greenish-black
Conc.H_2SO_4	Dark brown	Deep bluish-black	Green
Conc.HNO_3	Yellowish brown	Black	Light-green
Conc.HCL	Greenish yellow	Light-blue	Yellowish-green
5% Iodine	Yellowish brown	Deep blue	Deep green
NaOH Soln.	Brownish black	Black	Greenish-black

Table 5: Fluorescence characters of powdered Stem

Particulars of treatment	Under Visible light	Under UV light	
		Short Wavelength	Long Wavelength
Water	Brownish-black	Light black	Greenish-black
5% Picric acid	Yellowish-black	Bluish-black	Yellowish brown
NH_3 solution	Greenish-black	Black	Greenish-black
Methanol	Brown	Black	Greenish-black
Acetic acid	Yellowish-brown	Bluish-black	Light yellowish-black
Conc.H_2SO_4	Dark-brown	Dark-black	Greenish-black
Conc.HNO_3	Yellowish-brown	Light-black	Yellowish-green
Conc.HCL	Purple	Light-black	Brownish-green
5% Iodine	Blackish-brown	Light-black	Light greenish-black
NaOH Soln	Brownish-black	Dark-black	Greenish-black

Table 6: Fluorescence characters of powdered Root

Particulars of treatment	Under Visible light	Under UV light	
		Short Wavelength	Long Wavelength
Water	Light-brown	Light bluish-black	Brown
5% Picric acid	Light-brown	Deep blue	Yellowish brown
NH_3 solution	Dark-brown	Bluish-black	Greenish-black
Methanol	Light-brown	Black	Light brownish-black
Acetic acid	Yellowish brown	Black	Greenish-black
Conc.H_2SO_4	Dark brown	Black	Greenish-black
Conc.HNO_3	Brown	Black	Dark-green
Conc.HCL	Brownish-black	Black	Greenish-black
5% Iodine	Brownish-black	Black	Greenish-black
NaOH Soln	Dark brown	Black	Greenish-black

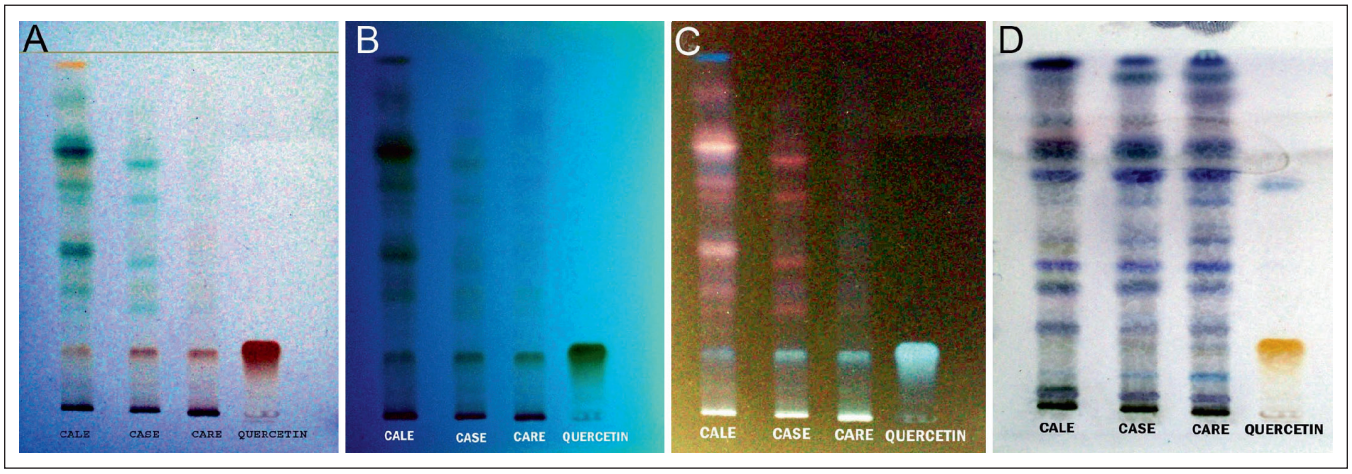

Figure 2: TLC profile of the alcoholic extract of various parts of *Coleus amboinicus.* A. visible light, B. UV 366, C. UV 254, D. After spraying with Anisaldehyde- sulphuric acid reagent. CALE- *Coleus amboinicus* leaf extract, CASE- *Coleus amboinicus* stem extract, and CARE- *Coleus amboinicus* root extract.

CONCLUSION

The data generated from the present studies would help in the authentication of various parts of *Coleus amboinicus*. The microscopic features and the quantitative standards would be useful for laying down pharmacopoeial standards. The different spots observed in TLC would be definitely useful in deciding the purity and quality of the drug. Morphology as well as various pharmacognostic aspects of different parts of the plant were studied and described along with phytochemical, physicochemical and TLC studies in authentication for quality control.

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Pharmacognostical and Phytochemical Evaluation of Leaves of *Bauhinia variegata* Linn.

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ABSTRACT

Bauhinia variegata Linn. (Caesalpiniaceae) syn: *kovidara* and *kachnar* is a medium sized deciduous tree generally found in sub Himalayan region of India. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. The present work was undertaken to establish the pharmacognostic and phytochemical standards along with HPTLC densitometric analysis of leaves for evaluating the plant material. The macro and microscopical studies indicated presence of pulvinus base with grooved petiolate leaf, emarginated apex, rough surface with 11-13 reticulate and palmate-divergent venation with scattered prismatic calcium oxalate crystals throughout the transverse section. Physiochemical studies revealed that total ash is 9.42%, acid insoluble ash is 5.72%, water-soluble extractive value is 3.30% and loss on drying at 105 °C is 6.27%. Preliminary phytochemical analysis revealed the presence of alkaloid, tannin, flavonoid, steroid, triterpenoid and saponin in different extracts. HPTLC fingerprinting for flavonoids revealed presence of two flavonoids rutin and kaempferol. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Key words: *Bauhinia variegata*, Leaf microscopy, Physico-chemical constants, Phytochemical, HPTLC finger print

INTRODUCTION

About 250 species of Bauhinia grow in the tropical regions of the world. It includes shrubs, trees and vines that are frequently planted for their showy flowers and ornamental foliage.[1] *Bauhinia variegata* Linn. is native to south eastern Asia and grows throughout India and China. It is most commonly cultivated in India.[2] *Bauhinia variegata* Linn. (Caesalpiniaceae) is a medium sized deciduous tree, known as (Sanskrit) *kanchanara*, (Hindi) *kovidara* and (Marathi) *raktakanchan*. Almost all parts are used in traditional medicine for the treatment of various ailments like asthma, ulcer, leprosy, piles, snake bite and liver complaints[3] and its extracts have been found to have antibacterial and antifungal ativity.[4] It is also used in fever, diarrhoea, dysentery, hemorrhoids, piles, edema, skin diseases, wound healing, obesity, stomatitis, dyspepsia, flatulence and as tonic, astringent, laxative, anthelmintic, antileprotic, antigoitrogenic, antitumor, and carminative.[5] The leaves of other bauhinia species are reported to have antiophidian,[6] antidiabetic,[7] antimalarial,[8] and antioxidant potential.[9]

Previously reported phytochemical constituents from the plant are lupeol, β-sitosterol, tannins, kaempferol-3-glucoside,[10] amides, carbohydrates, reducing sugars, crude protein, vitamin C, fibers,[11] calcium, phosphorus,[12] rutin, quercetin, quercitrin, apigenin, apigenin -7-O-glucoside,[13] dotetracont-15-en-9-ol and heptatriacontan-12,13-diol.[14] Inspite of its abundant uses, the phytochemical standards of *Bauhinia variegata* leaves have not been reported.

The present investigation deals with the qualitative and quantitative pharmacognostical and phytochemical evaluation of the leaf material.

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DOI: 10.5530/pj.2011.24.9

MATERIALS AND METHODS

Plant Material

The plant material was collected from the natural park of the Mehsana district, Gujarat, India in the month of February 2009. The plant was authenticated by Pro. Y. B. Dabgar, Head , Department of Botany, Shri C. L. Parikh & R. R. Mehta Science College, Palanpur, Gujarat, India and a voucher specimen (No. SSPC/COG/05/2009) was deposited in Department of Pharmacognosy, Shri Sarvajanik Pharmacy College, Mehsana, Gujarat, India.

Pharmacognostical studies

Morphology

Morphological studies such as shape, size, apex, surface, base, margin, venation, taste and odour of leaves were carried out.

Microscopy

Microscopical studies were carried out using Nicon Labphot-2 instrument (Japan). The transverse sections with average thickness of 10-12 µm were taken with the help of rotary microtome. Dewaxing of the sections was performed by customary procedure.[15] The section was stained with toluidine blue as per the method.[16] Since, toluidine blue is a polychromatic stain, it rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Wherever necessary, sections were also stained with saffranin, fast-green and iodine potassium iodide reagents (for starch).

As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number and vein termination number were studied by taking paradermal sections as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling off by partial maceration employing Jeffrey's maceration fluid[17] as shown in table 1.

Physico-chemical constants

Physicochemical constants of the leaf such as the total ash, acid insoluble ash, water soluble ash and loss on drying were calculated based upon standard procedures.[18]

Phytochemical analysis

For preliminary phytochemical studies, 300 g of powdered material was extracted in soxhlet apparatus with petroleum ether, chloroform, methanol and water. Extracts were dried in rotary evaporator and weighed. The extractive values for each extract is shown in table 2. The presence of various phytoconstituents like steroids and triterpenoids (Liberman and Burchard test), alkaloids (Dragandroff's test), tannin (Ferric chloride test), flavonoid (Shinoda test), Sugar (Fehling solution test) were detected by usual method prescribed in standard text.[19,20]

Densitometric HPTLC analysis for flavonoids

A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. 50% hydroalcoholic and methanolic extract of *Bauhinia variegata* Linn. leaves were dissolved with HPLC grade methanol 10 mg/ml. The solutions were sonicated for 10 min and used for HPTLC analysis. Then, 10 µl of the samples were loaded as 5 mm band length in the 10 × 10 Silica gel $60F_{254}$ TLC plates using Hamilton 254 syringe and Desaga Sarstedt-gruppe AS 30 instrument. The sample loaded plate were kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase along with 2000 µg/ml rutin and 200 µg/ml kaempferol standard solutions. The plates were developed in the Ethyl acetate-Butanol-Formic acid-Water (10:6:2:2 v/v/v/v) for rutin (Rf 0.48) and Toluene-Ethyl acetate-Methanol-Formic acid (6:3:0.2:0.4 v/v/v/v) (Rf 0. 56) for kaempferol up to 90 mm. The developed plate was dried using hot air to evaporate solvents from the plate and kept in Photo-documentation chamber (Desaga) and captured the images at UV254. Finally, the plates were fixed in scanner stage and scanned at 380 nm and 254 nm for rutin and kaempferol respectively.

Table 1: Leaf constants of the *Bauhinia variegata* Linn. leaf

Leaf constant	Values
Palisade ratio	4.8
Stomatal index	5.6
Vein-islet number	5.8
Vein termination number	3.7

Table 2: Extractive values of the *Bauhinia variegata* Linn. leaf

Extract	Colour	% w/w
Petroleum ether	Yellowish brown	3.41
Chloroform	Brownish black	1.72
Methanol	Brown	8.76
Aqueous	Greenish brown	7.25

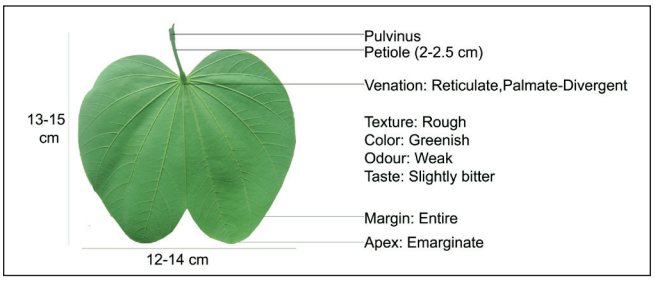

Figure 1: Leaf of *Bauhinia variegata* Linn.

RESULTS AND DISCUSSION

Organoleptic characters

Colour of *Bauhinia variegata* Linn. leaves is green on both side when fresh and brown in dry state. Size and shape is 13-15 × 12-14 cm, long as broad as or rather broader than long, cleft 1/4 to 1/3 of the way down into 2 obtuse lobes, pulvinus base with grooved petiolate, linear-lanceolate with entire margin with soft stipules. Apex of the leaf is broad and emarginated. The surface of leaf is rough surface with 11-13 reticulate, palmate-divergent venation as shown in figure 1. Taste is slightly bitter and having weak odor.

Microscopy

Transverse section of the leaf (Figure 2)

Lamina

It shows dorsi-ventral nature; more densely covered upper epidermis with cuticle than lower epidermis and made up of thin walled tangentially elongated rectangular cells. Mesophyll in the lamina shows the presence of 2-3 layers of palisade parenchyma below the upper epidermis and spongy parenchyma above the lower epidermis as shown in figure 2 (B). Scattered prismatic calcium oxalate crystals are present throughout the mesophyll.

Midrib

Ventral side of the midrib is slightly concave. Shape of the middle vein portion in the TS is oblong but elongated tapered at ventral side and also shows the irregularities on its lower epidermis. Midrib contains 'U'- shape well developed vascular bundle at the centre surrounded by sclerenchyma (pericyclic lignified fibrous tissue in a band). Vascular bundle shows the presence of the xylem at the upper side and phloem at the lower side and well developed collenchyma below the upper epidermis and above the lower epidermis with scattered prismatic calcium oxalate crystals.

TS of the leaf also show the presence of unicellular, 3-5 celled multicellular uniseriate- cuticlerised covering trichomes as well as unicellular sessile and unicellular head with unicellular stalcked glandular trichomes. Trichomes are more prominent on the lower epidermis than upper epidermis.

Powder microscopy

The powder microscopy showed the presence of 142.8-199.92 µm long multicellular uniseriate trichomes, 57.12 µm in diameter prismatic calcium oxalate crystals, lignified annular and spiral xylem vessels, portion of epidermal cells with anomocytic stomata as shown in figure 3.

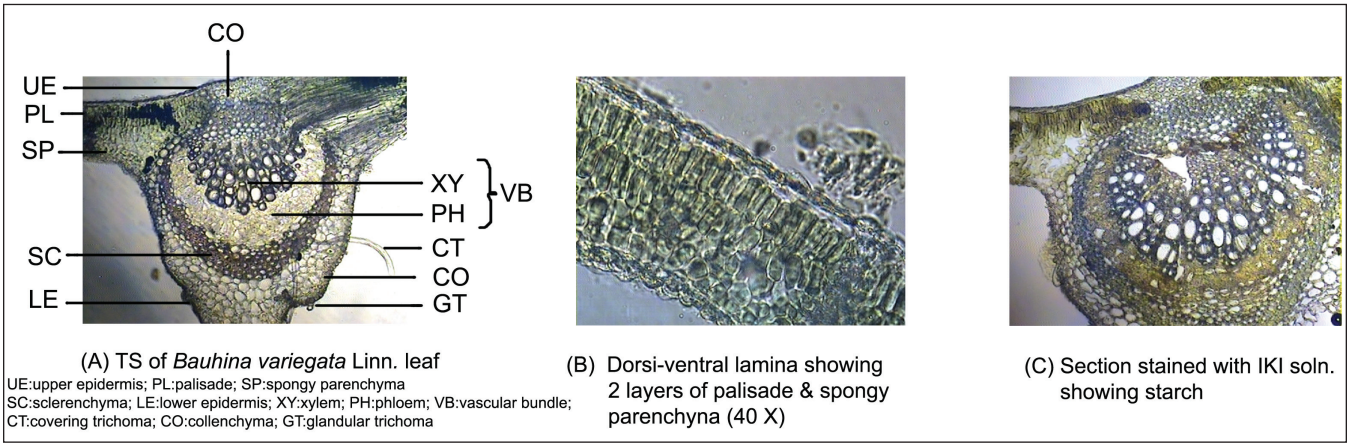

Figure 2: Leaf structure of *Bauhinia variegata* Linn.

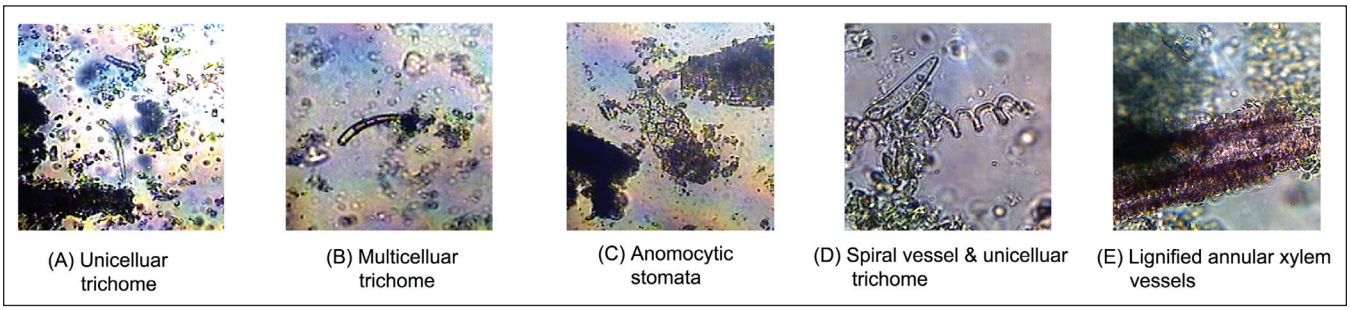

Figure 3: Powder microscopy of leaves of *Bauhinia variegata* Linn.

Physico-chemical parameters

The percentage of total ash, acid-insoluble ash, water soluble ash and loss on drying are tabulated in table 3.

Qualitative phytochemical analysis

The results demonstrated presence of alkaloids, flavonoids, saponin, tannins, steroids and sterol, triterpenoids and sugar in the leaf of *Bauhinia variegata* Linn. The presences of various phytoconstituents in various organic and aqueous extracts are summarized in table 4.

Quantification of rutin and kaempferol

Both extracts of the plant developed a characteristic HPTLC fingerprinting for rutin and kaempferol as shown in figure 4. It gave a distinct spot at Rf value 0.48 and 0.56 for rutin and kaempferol respectively as shown in table 5. The peak purity was assessed by comparison of overlay spectra of standards and both the extracts as shown in figure 5. The linear regression equation was Y = 1060 + 151.5*X and the correlation coefficient (R^2) was 0.993 for rutin and Y = 1576 + 895.2*X and the correlation coefficient (R^2) was 0.992 for kaempferol.

Quantification of rutin and kaempferol in both extracts shows that the sample of 50% hydroalcoholic extract (1.114 mg/100 g) contains more rutin than the sample from the methanolic extract (0.075 mg/100 g) whereas the sample of 50% hydroalcoholic extract (0.193 mg/100 g) contains more kaempferol than the sample from the methanolic extract (0.014 mg/100 g).

CONCLUSION

A variety of standardization parameters like morphological, microscopical, physico-chemical, phytochemical and chromatographic characterization were studied and generated

Table 3: Physicochemical constants of the *Bauhinia variegata* Linn. leaf

Sr. no.	Parameter	% w/w
1	Total Ash	9.42
2	Acid insoluble ash	5.72
3	Water soluble ash	3.30
4	Loss on drying	6.27

Table 4: Qualitative phytochemical analysis of the *Bauhinia variegata* Linn. leaf

Constituents	Pet.ether	Chloroform	Methanol	Aqueous
Alkaloids	–	+	–	–
Coumarins	–	–	–	–
Flavonoids	–	–	+	–
Anthraquinone	–	–	–	–
Saponin	–	–	–	+
Tannins	–	–	+	+
Steroids and sterol	+	+	+	–
Triterpennoids	–	–	+	+
Sugar	–	+	+	+
Fixed oils	–	–	–	–

+ Presence; - Absence

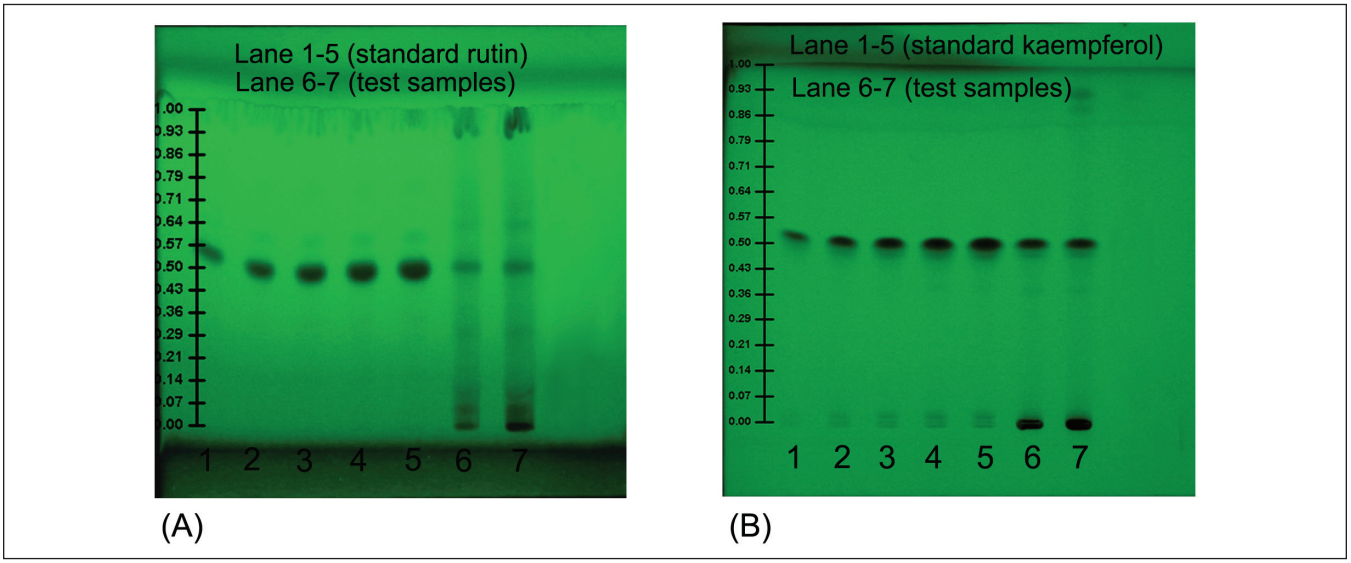

Figure 4: TLC plate at 254 nm for (A) rutin and (B) kaempferol

Table 5: Densitometric HPTLC analysis of the *Bauhinia variegata* Linn. leaf

Extract	Rutin			Kaempferol		
	Rf value	Area	Concentration (mg/100 g)	Rf value	Area	Concentration (mg/100 g)
50% Hydroalcoholic	0.48	2048	1.114	0.56	3379.36	0.193
Methanolic	0.47	1907.05	0.075	0.56	3314.10	0.014

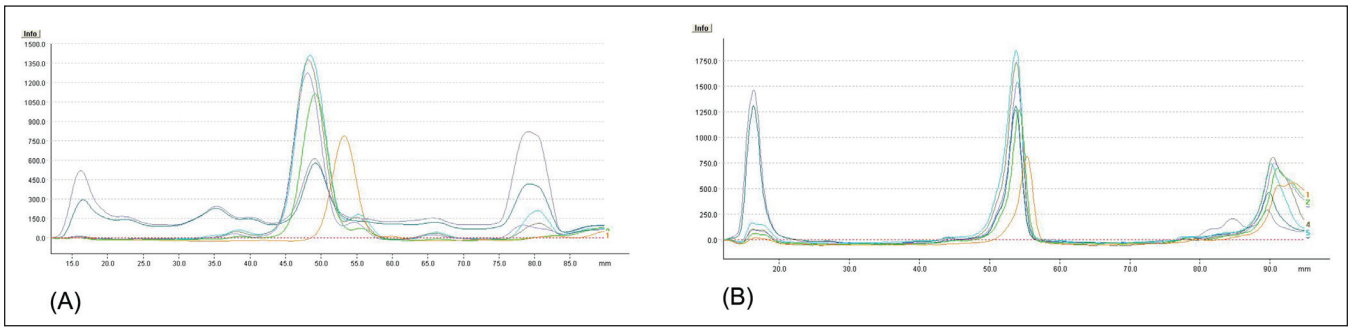

Figure 5: Overlay spectra of 50% hydroalcoholic and methanolic extracts of leaves of *Bauhinia variegata* Linn. over standard (A) rutin and (B) kaempferol

data could be useful for the assessment of quality of plant material, and also to check the adulteration and substitution etc., for future reference.

The pharmacognostic study of *Bauhinia variegata* Linn., have furnished a set of qualitative and quantitative standards that may substantiate to ascertain it's identity and to establish the quality and purity of this plant material in closely related species. Densitometric HPTLC analysis may serve a supplementary data for the standardization of the drug, particularly of different batches. This could also serve in the establishing data for preparation of monograph of this plant.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Yogesh B. Dabgar, Head, Department of Botany, Shri C. L. Parikh & R. R. Mehta Science College, Palanpur, Gujarat, India for authentication of plant sample.

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Profile of Elemental Composition of *Oroxylum indicum* L.(Vent.) Collected from Different Geographical Regions of India

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ABSTRACT

Mineral content was quantified in *Oroxylum indicum* L.(Vent.) collected from two different geographic regions of India viz. Western Ghats (Maharashtra) and Northern Uttar Pradesh. The main aim of this study was determination of elemental composition in different parts of the plant *Oroxylum indicum* L.(Vent.), which is extensively used in Ayurvedic preparations. Specific parts (leaves, stem and root) often used in Indian ayurvedic system were analyzed for 10 elements viz Cu,Na,Ca,Cr,Mn,Fe,Ni,Cd,Zn and Pb by employing ICPES techniques. All of the detected values for metallic elements in plant studied here were found to be below the WHO permissible levels. The elemental concentration in different part of medicinal plants and their biological effects on human beings are discussed.

Key words: *Oroxylum indicum* L.(Vent.), ICPES, elemental composition.

INTRODUCTION

The curative properties of plants have been well documented in ancient Indian literature. The different parts of the plant are used as ingredients in several medicinal preparations in different systems of medicine including Ayurveda. The importance of herbal medicines in the health care system of the larger section of the world's population, the developing countries, is also an undeniable fact. They form and inseparable part of the traditional systems of medicine and in many cases bridge the gap between the availability of and demand for modern medicine. World Health Organization estimates that about 80% of the population living in rural areas use or depend on plant based medicinal preparations for preventive and curative health care.

Epidemiological studies over the past decades have documented the importance of trace elements in human health and disease. Prompted by this development the pharmaceutical companies have been marketing as general tonics a variety of formulations containing combinations of different trace element contents .Various medical studies over the past decades have focused on the clinical significance of trace elements in human health and disease. Trace quantities of these elements are essential for enzyme catalyzed biological processes in plants. Consequently, the presence of these elements attributes medicinal properties to the plant. It is often observed that these elements are present at varying levels of concentration in different parts of the plants such as roots, stem, leaves etc. It is also observed that these elements concentration vary depending upon the geographical location of the plant.

It has been reported that whatever is consumed as medicine could cause metabolic disturbances subject to the allowed upper and lower limits of trace elements.[1] Both the deficiency and excess of essential micronutrients and trace elements of toxic metals may cause serious effects on human health.[2,3]

According to WHO, medicinal plants should be checked for the presence of heavy metals. It is an established fact that the overdose or prolonged ingestion of the medicinal plant leads to the chronic accumulation of different elements which cause various health problems.[4,5] In this context elemental contents of the medicinal plants are very important and need to be screened for their quality control.[6]

For the present study, *Oroxylum indicum* L.(Vent.) a medicinal plant, belonging to the family *Bignoniaceae* was selected. It is

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DOI: 10.5530/pj.2011.24.10

a medium sized, deciduous tree found in India in Eastern and Western Ghats and North East regions. It is an important herb in ayurvedic medicine and indigenous medical system for over thousands of year.[7] As the plant is used in many medicinal formulations like Chyawanprash, dashamoolarishtha[8] etc, a detailed study is carried out to analyze the concentration levels of elements. The stem bark and root bark possess anti allergic properties and are used in treating allergic diseases urticaria, jaundice, asthma, sore throat, laryngitis and measles.[9] As the quantitative profile of a plant medicine may vary depending upon geological and ecological factors, the same species is collected from two different locations viz., Western Ghats and Northern region of India to compare the various elemental concentrations. The elemental concentration in different part of medicinal plant and their biological effects on human beings are discussed.

MATERIALS AND METHODS

Sample collection

Whole plant parts of *Oroxylum indicum* L.(Vent.) were collected during the flowering season from two different geographical regions viz., Western Ghats (Village – Pophali, Kumbharli Ghat near Chiplun, dist Raigarh , Maharashtra) and Khiri village, dist Lakhimpur, Northern U.P.

The plants were identified and authenticated at Blatter's herbarium, St. Xavier's College, Mumbai, (Accession No 54436). The plants collected from different regions were sorted out and individual plant parts were separated.

Sample Preparation

Plant parts were washed with de ionized water and oven dried at 40 °c for four days and then subjected to grinding for powder formation. The powder was stored in air tight glass containers and used for further analysis.

Digestion

Two gram powder of each plant part was dissolved in nitric acid and heated until the reddish brown fumes disappear. Perchloric acid was then added to the above solution and heated for 5 min. This was followed by addition of aqua regia and heated .The volume was then made up to 250 ml in a standard flask by adding de ionized water.

Estimation of elements was carried out using Inductively Coupled Plasma - Atomic Emission Spectrometer (Model: ARCOS from M/s. Spectro, Germany)

RESULTS AND DISCUSSION

The results presented in table 1 exhibit that the various plant parts of *Oroxylum indicum* L.(Vent.) are a good source of trace and major elements.

Copper: Copper content was found to be variable among the plant parts in the two plants, ranging from 19.16 ppm to 55.69 ppm, with *Oroxylum indicum* L.(Vent.) stem sample from Western Ghats recording the highest level. This is in accordance with reports[10] which state that range of copper content in 50 medicinally important leafy materials growing in India is in the range of17.66 ppm to 56.3 ppm. Copper (Cu) is an essential redox-active transition element that play vital role in various metabolic processes. Being toxic, its quantity should be mentioned very low. It is well known that the high content of transition metal like Cu catalyzes the formation of hydroxyl (OH) radicals, hence their excess quantity can cause oxidative stress in plants and consequently increase the antioxidant response.[11]

It is essential to the human body since it forms a component of many enzyme systems, such as cytochrome oxidase, lysyl oxidase and an iron-oxidizing enzyme in blood. The observation of anemia in copper deficiency is probably related to its role in facilitating iron absorption and in the incorporation of iron in hemoglobin. However copper deficiency in humans is a rare occurrence. Copper could be toxic depending on the dose and duration of exposure.[12] The permissible limit set by FAO/WHO[13] in edible plants

Table 1: Concentration of elements in *Oroxylum indicum* L.(Vent.) collected from different regions of India

Elements Analysed	Western Ghats			Uttar Pradesh		
	Root	Stem	Leaves	Root	Stem	Leaves
Cu	45.45	55.69	19.16	33.37	21.47	23.93
Na	279.22	272.98	840.84	152.27	310.16	1685.32
Ca	4513.49	7708.04	9311.72	3275.90	7017.47	10657.53
Cr	nd	nd	nd	nd	nd	nd
Mn	46.70	12.49	26.26	28.26	14.73	86.62
Fe	2288.34	233.77	293.31	1347.61	402.90	1129.11
Ni	nd	nd	nd	nd	nd	nd
Cd	nd	nd	nd	nd	nd	nd
Zn	27.85	59.94	28.00	21.29	17.60	27.79
Pd	nd	nd	nd	nd	nd	nd

(All values in ppm -- microgram per gram; nd—values less than 0.01 ppm)

FIGURES: Elemental concentration in *Oroxylum indicum* L.(Vent.) collected from different regions of India.

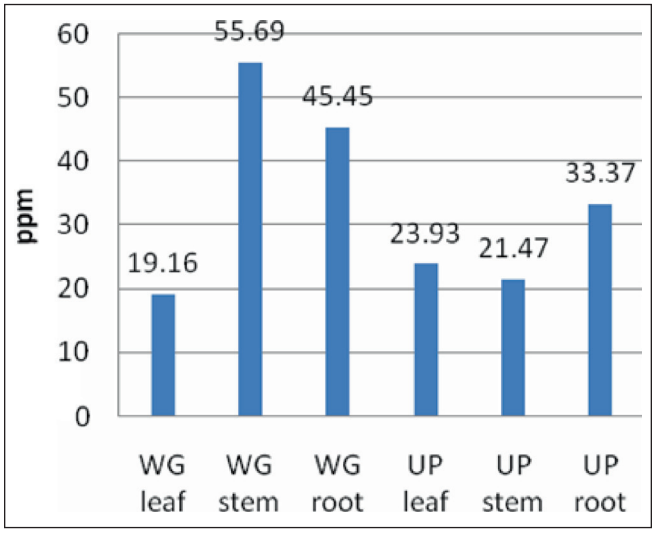

Figure 1: Concentration of Copper in different plant parts collected from different regions of India (WG – Western Ghats;UP –Uttar Pradesh)

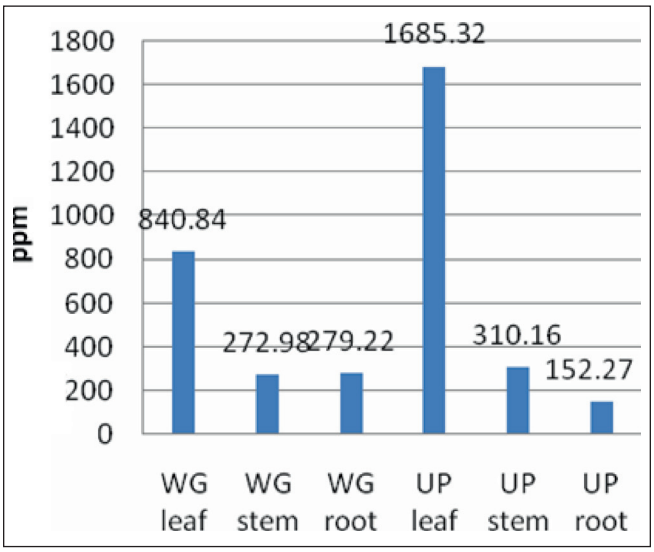

Figure 2: Concentration of Sodium in different plant parts collected from different regions of India (WG – Western Ghats; UP – Uttar Pradesh)

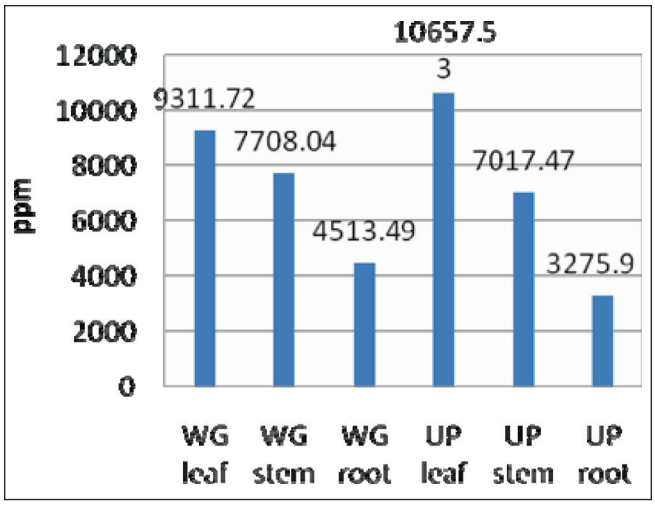

Figure 3: Concentration of Calcium in different plant parts collected from different regions of India (WG –Western Ghats; UP –Uttar Pradesh)

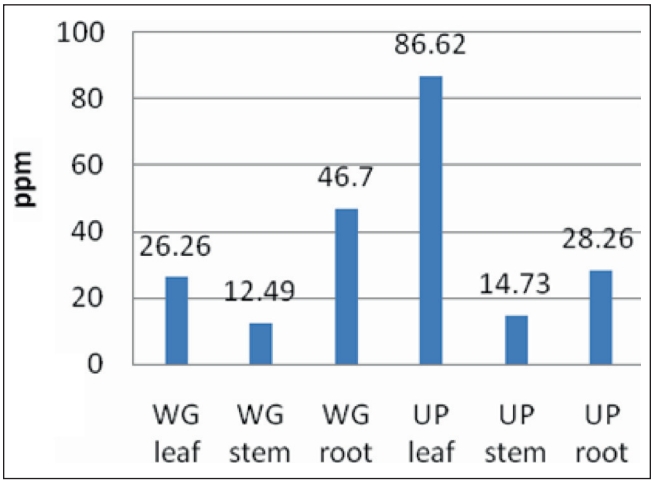

Figure 4: Concentration of Manganese in different plant parts collected from different regions of India (WG –Western Ghats; UP –Uttar Pradesh)

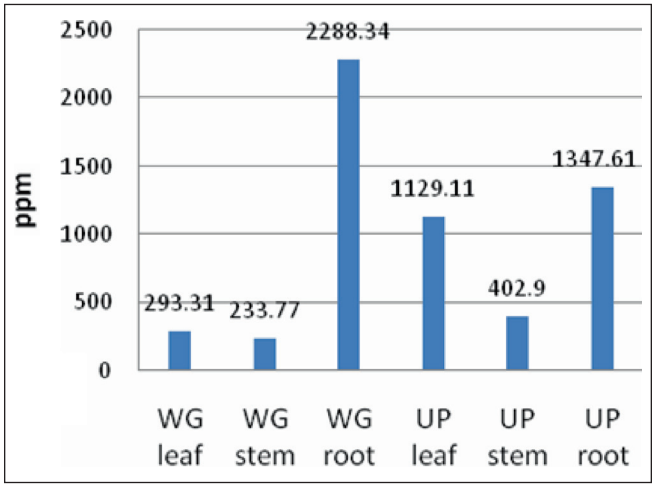

Figure 5: Concentration of Iron in different plant parts collected from different regions of India (WG –Western Ghats; UP –Uttar Pradesh)

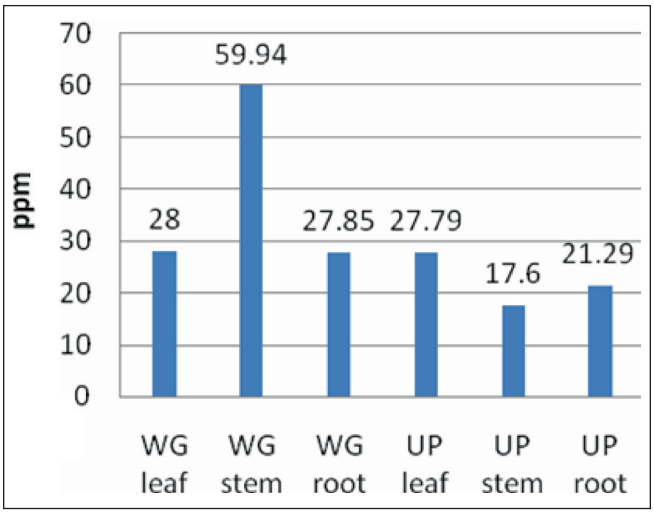

Figure 6: Concentration of Zinc in different plant parts collected from different regions of India (WG –Western Ghats; UP –Uttar Pradesh)

is 3.00 ppm. However for medicinal plants the WHO limits have yet not been established for Cu.[14]

Sodium: Sodium is essential to all living organisms. Na is an important element for the maintenance of acid–base equilibrium and of osmotic pressure of body fluids.[15] Concentration of Sodium element was observed to be much higher in leaves from both plants than the concentration in other plant parts. The lowest amount, 152.27 ppm was found in roots of *Oroxylum indicum* L.(Vent.) from U.P and highest amount was found in leaf of *Oroxylum indicum* L. (Vent.) from U.P.

Calcium: Calcium is an important trace element because of its role in bones, teeth, muscular system and heart functions.[16] It is required for absorption of dietary Vit. B, for synthesis of neurotransmitter acetylcholine and is also required for activation of enzyme pancreatic lipase.[17] It is observed that amongst all the metals studied in the analyzed samples, calcium accumulation is highest in all parts than the concentration of other metals. Maximum concentration is found in leaves than the other plant parts. U. P root sample showed the lowest 3275.90 ppm while U.P. leaf has the highest amount i.e. 106.57 ppm.

Chromium: Chromium is known to regulate carbohydrate, nucleic acid and lipoprotein metabolism and it also potentiates insulin action.[18] Chronic exposure to Cr may result in liver, kidney and lung damage.[19] Chromium also acts as an activator of several enzymes. Deficiency of chromium decreases the efficiency of insulin and increases sugar and cholesterol in the blood. Chromium deficiency can cause an insulin resistance, impair in glucose tolerance and may be a risk factor in atherosclerotic disease. The permissible limit for chromium as set by FAO/WHO[13] in edible plants is 0.2 ppm. However the permissible limit for medicinal plants has yet not been set. The chromium concentration in all the samples studied was below the permissible levels.

Manganese: The highest concentration of manganese found in the sample, UP leaf was 10657.53 ppm and the least was found in W. Ghats stem i.e. 12.49 ppm. The permissible limit for Mn is 2 ppm in edible plants. The permissible limits for Mn in medicinal plants have yet not been set.

Iron: The permissible level set by WHO for Iron in edible plants was 20 ppm. Iron is important for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in human body. In all the samples studied, the amount of iron accumulated is much higher than the permissible levels. Studies suggest that the intake of Iron in higher concentration is hazardous to health. W.Ghat root sample showed highest concentration 2288.34 ppm and lowest concentration was found in W.Ghat stem, 233.77 ppm.

Nickel: Nickel is considered to be highly mobile element within a plant. Accumulation of Ni takes place only in the leaves.[20] Ni toxicity in human is not very common occurrence as its absorption by the body is very low.[21] The permissible limit for nickel set by WHO in edible plants was 1.63 ppm and the amount of nickel concentration in all the samples analyzed was below the permissible level. The permissible limits for medicinal plants have yet not been set.

Cadmium: Cadmium is toxic metal having functions neither in human body nor plants.[22] Accumulation of Cd in kidney leads to high blood pressure and renal diseases. Its accumulation also leads in damaging the nerve cells, inhibition of release of acetylcholine and activation of choline esterase enzyme, resulting in a tendency for hyperactivity of the nervous system.[23]

The permissible level (WHO) for cadmium in edible plants was 0.21 ppm and for medicinal plants is 0.3 ppm.[14] The amount of cadmium concentration in all the samples analyzed was found to be within the permissible limits. This may be due to low level of cadmium present in the available soil for plant growth.

Lead: Exposure to increased concentrations of lead is a health hazard. The permissible limit for lead set by FAO/WHO in edible plants was 0.43 ppm. The amount of lead concentration in all the samples analyzed was in minimal amount and well below the permissible level.

Zinc: Zinc is essential to all organisms and has an important role in metabolism, growth, development and general wellbeing. It is an essential co-factor for a large number of enzymes in the body. Zinc deficiency leads to coronary heart diseases and various metabolic disorders. Highest amount found in W. Ghats plant stem 59.94 ppm and lowest in17.60 ppm in U.P. stem.

The results above indicated that the plants contain large amounts of nutrients and are rich in Fe, Copper, Ca and Na. The abundance of Fe, Ca and Cu, in the result of this analysis, was in agreement with previous findings that these three elements represent the most abundant metal constituents in plants.[24,25] High contents of Ca are important, because of its role in human and studied plants show satisfactory level of Ca accumulation

CONCLUSIONS

In view of above facts, the medicinal plant, *Oroxylum indicum* L.(Vent.) studied is a source of biologically important elements, which may play a part in the observed therapeutic use of this plant. Ayurvedic formulations do demonstrate

significant success in treatment of many diseases. These medicines contain trace elements whose activity has an impact on its overall pharmacological action. There is no direct link that has been established between elemental content and curative capability of the plant. But such studies will help us to understand the pharmacological action of the herb and thus provide the vital link between the two. The data obtained in the present work will be helpful in the synthesis of new Ayurvedic drugs which can be used for the control and cure of various diseases. However, in order to develop a stronger basis for appreciating the curative effects of medicinal plant, *Oroxylum indicum* L.(Vent.) there is a need to study the effect of soil and climatic conditions on the elemental contents of this medicinal plant. Medicinal plant *Oroxylum indicum* L.(Vent.) is rich in metals Fe, Copper, Ca and Na and it is expected that plants with high contents of the above-mentioned macro and micronutrients, might play an important role in maintenance of human health. Also, all of the detected values for metallic elements in plant studied here are below the WHO permissible levels and may not constitute a health hazard for consumers.

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Pharmacognostical, Physicochemical and Phytochemical Studies of Some Marketed Samples of Roots used in Ayurvedic Medicines

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ABSTRACT

Background: Kuth (*Saussurea lappa*), Nisotha *(Operculina turpethum),* Akarakara *(Anacyclus pyrethrin)* and Chitrak (*Plumbago zeylanicum)* are some common plants used in Ayurvedic system of medicines and herbal drugs. **Objective:** The objective of the present study is to evaluate the quality of the samples of same plant marketed into different area, on the standardization parameters given in Ayurvedic pharmacopoeia. **Methods:** Three different marketed samples of these roots were subjected to pharmacognostic, physicochemical and phytochemical analysis and results were compared to the standards given in Ayurvedic pharmacopoeia. **Results:** Variations were found in physicochemical and phytochemical parameters of two samples in each case of *Saussurea lappa, Operculina turpethum, Anacyclus pyrethrin* and of one sample in case of *Plumbago zeylanicum*. Pharmacognostical parameters were found same as given in Ayurvedic pharmacopoeia for all the samples. **Conclusions:** The outcome of the study suggested that there is a lot of difference in the quality of the same drug marketed in different parts of country which may also cause variation in products prepared from them. These findings may be very useful for the identification of the species which may be useful to pharmaceutical industries for the quality control of the commercial samples.

Keywords: Ayurvedic Pharmacopoeia, Commercial sample, Quality control.

INTRODUCTION

Kuth (*Saussurea lappa*), Nisotha (*Operculina turpethum*), Akarakara (*Anacyclus pyrethrin*) and Chitrak (*Plumbago zeylanicum*) are some common plants used in Ayurvedic system of medicines and herbal drugs.[1,2,3,4] Kuth is used for anti-inflammatory, anti-ulcer, anticancer and hepatoprotective activities; Nisotha is an important ingredient of Ayurvedic formulation viz. Avipattikara churna used for the treatment of gastric ulcer, gastrointestinal related disturbances and also used in treatment of piles, tumors and jaundice.[5,6,7] It also reported to have hepatoprotective and antimicrobial activities.[8,9] Akarakara plant is used in traditional system of medicine as a tonic to the nervous system and also reported to have antibacterial, antidepressant and anti-inflammatory activities.[10] Chitrak is used against a number of ailments including skin diseases, diarrhea and leprosy. It also possesses antibacterial, antifungal, anti-carcinogenic, antitumor properties.[11] These plant drugs are available in local market of many cities in Uttar Pradesh, from where they are utilized by local people as home remedies and by small scale Ayurvedic drug manufacturers. These Plant materials may vary in their quality and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal plant.[12] This difference may cause batch to batch variation or also may cause city to city variation in quality, safety and efficacy of same formulation. The objective of the present study is to evaluate the quality of the samples of same plant marketed into different area, on the standardization parameters given in Ayurvedic pharmacopoeia.

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DOI: 10.5530/pj.2011.24.11

MATERIALS AND METHODS

Sample collection

The samples of all four drugs were purchased from three different locations and labeled properly as per [table 1].

Authantication

Samples were authenticated by Dr. N. K. Dubey, Professor, Department of Botany, BHU, Varanasi and a voucher specimen is preserved in herbarium section for future reference.

Organoleptic properties

Organoleptic properties were evaluated including appearance, size, color, taste and odour following the method described by *Wallis et. al,* 1989.[13] For determining the odor of an innocuous material, small portion of the sample was placed in the beaker of suitable size, and examined by slow and repeated inhalation of the air over the material. If no distinct odor was perceptible, the sample was crushed between the thumb and index finger, between the palms of the hands, using gentle pressure or if the material was known to be dangerous, by other suitable means such as pouring a small quantity of boiling water onto the crushed sample placed in a beaker. First, the strength of the odor was determined (none, weak, distinct, strong) and then the odor sensation (aromatic, fruity, musty, moldy, rancid, etc.) was studied. Taste was distinctively classified as aromatic, pungent, sweet, sour, astringent, mucilaginous, or bitter.

Table 1: Location of sample procurement and coding

Sample Name	Location of Collection	Code for labeling
Kuth (*Saussurea lappa*)	Dinanath Gola Market, Varanasi	SL1
	Buddha Bazaar, Moradabad	SL2
	Chauk market, Jhansi	SL3
Nisotha (*Operculina turpethum*)	Dinanath Gola Market, Varanasi	OT1
	Buddha Bazaar, Moradabad	OT2
	Chauk market, Jhansi	OT3
Akarakara (*Anacyclus pyrethrin*)	Dinanath Gola Market, Varanasi	AP1
	Buddha Bazaar, Moradabad	AP2
	Chauk market, Jhansi	AP3
Chitrak (*Plumbago zeylanicum*)	Dinanath Gola Market, Varanasi	PZ1
	Buddha Bazaar, Moradabad	PZ2
	Chauk market, Jhansi	PZ3

Microscopic study

All samples were cleaned and boiled separately. Their transverse sections were cut, stained, mounted and observed under microscope.[14]

Physicochemical analysis

Water soluble extractive value

Five gm. of the air-dried, coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaked frequently during the first 6 hours and allowed to stand for 18 hours. There often filtered rapidly evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and was dried at 105 °C and weighed. Percentage of water soluble extractive was calculated with reference to the air dried drugs.

Ethanol soluble extractive value

Five gm. of the air dried coarsely powdered drug was macerated with 100 ml of ethanol of the specified strength in a closed flask for 24 hours, shaked frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter rapidly taking precautions against loss of ethanol, evaporated 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish and was dried at 105°C, and weighed. Percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Total Ash value

Accurately 2 g of the air dried crude drug was weighed in a tarred platinum or silica dish and incinerate at a temperature not exceeding 450° until free from carbon and then cold and weighed again. Percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value

Accurately 2 g of air dried crude drug was weighed in a tarred platinum or silica dish and incinerated at temperature not exceeding 450°C until free from carbon and then cold and weighed again. Then the ash was boiled with 25 ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited, cold in desiccators and weighed. Percentage of acid insoluble ash was calculated with reference to the air-dried drug.[15]

Qualitative Phytochemical analysis

To detect the presence of various phytoconstituents in these samples, phytochemical investigation was performed.[16,17]

Thin layer chromatography

TLC of the alcoholic extract of OT1, OT2 and OT3 was developed on Silica gel 'G' plate using Toluene: Ethylacetate (9:1) as mobile phase. Vanillin-sulphuric acid reagent was

Table 2: Results of organoleptic properties test

Sample	Colour	Odour	Taste	Size and shape
S L1	Brown	None	Blunt	10-15 cm. long, 1.5 cm broad, thick , cylindrical
SL2	Brown	None	Blunt	5-8.5 cm. long, 1 cm broad, thick, cylindrical, hard
SL3	Grayish	None	Blunt	5-10 cm. long, 1.5 cm broad, thick, cylindrical hard.
OT1	Dull grey	None	Acrid	1-7 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles.
OT2	Brown	None	Acrid	1-10 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles, thin rootlets.
OT3	Dull grey	None	Acrid	1-15 cm long, 1 cm diameter, cylindrical, longitudinal wrinkles,
AP1	Dark brown	None	Pungent	8-10 cm long, tapering, hairy rootlets.
AP2	Dark brown	Slightly	Pungent	5-10 cm long, tapering, hairy rootlets. Aromatic
AP3	Grayish brown	None	Blunt	5-10 cm long, tapering, hairy rootlets.
PZ1	Brown	None	Acrid	10-15 cm long, 1.2 cm dia. cylindrical
PZ2	Brown	Disagreeable	Acrid	15-25 cm long, 1 cm dia., cylindrical
PZ3	Brown	None	Acrid	15-20 cm long, 1 cm dia. cylindrical.

used as detecting agent .Color, number and Rf values of spots were compared with the standards given in Ayurvedic pharmacopoeia.[18]

RESULTS

Organoleptic properties

The results of organoleptic evaluations are presented in [Table 2] and [Figure 1].

Microscopic study

Transverse section of root samples SL1, SL2 and SL3 showed the presence of cork, 3-5 layered wide, secondary phloem consisting of mostly storage parenchyma, modularly rays multi seriate, resin canals throughout as cavities, xylem, fibers, vessels and xylem parenchyma groups were found scattered in the center and inulin was observed in storage parenchyma. Transverse section of root samples OT1, OT2 and OT showed thin cork, consisting of 3-5 rows of brown cells, broad cortex consisted of clusters of parenchyma cells and resin canals. It was consisting of continuous circular zone of secondary phloem and dense secondary xylem, cleared radially in to wide four or five fan shaped segments by narrow xylem rays. The wide vessels, calcium oxalate crystals in prisms and rosettes shape were also observed. The starch grains were found scattered in cortex, phloem parenchyma, xylem parenchyma and medullary ray cells.

Transverse section of root samples of AP1, AP2 and AP3 showed cork consisting of tabular cells, many of which developed as sclerenchyma, a few sclerenchymatous cells also found scattered in secondary cortex; developed secondary phloem; cambium 2-5 layered; secondary xylem very wide consisting of xylem vessels, tracheids and xylem parenchyma; vessels pitted; medullary rays numerous, running straight, bi to tri and multi seriate; oleo-resinous schizogenous glands found scattered in secondary cortex, secondary phloem and medullary rays.

Figure 1: Photographs of SL1, OT1, AP1 and PZ1

Transverse section of root samples PZ1, PZ2 and PZ3 showed outer most layer cork with 5-6 rows of light brown cells, rectangular in shape; starch grains compactly packed in the cortex region, phloem well developed with phloem fibers. Groups of phloem fibers were present near the phloem. Cambium single layered, xylem was well developed with xylem vessels .Medullary ray was single to multilayered and loaded with simple to compound starch grains [Figure 2].

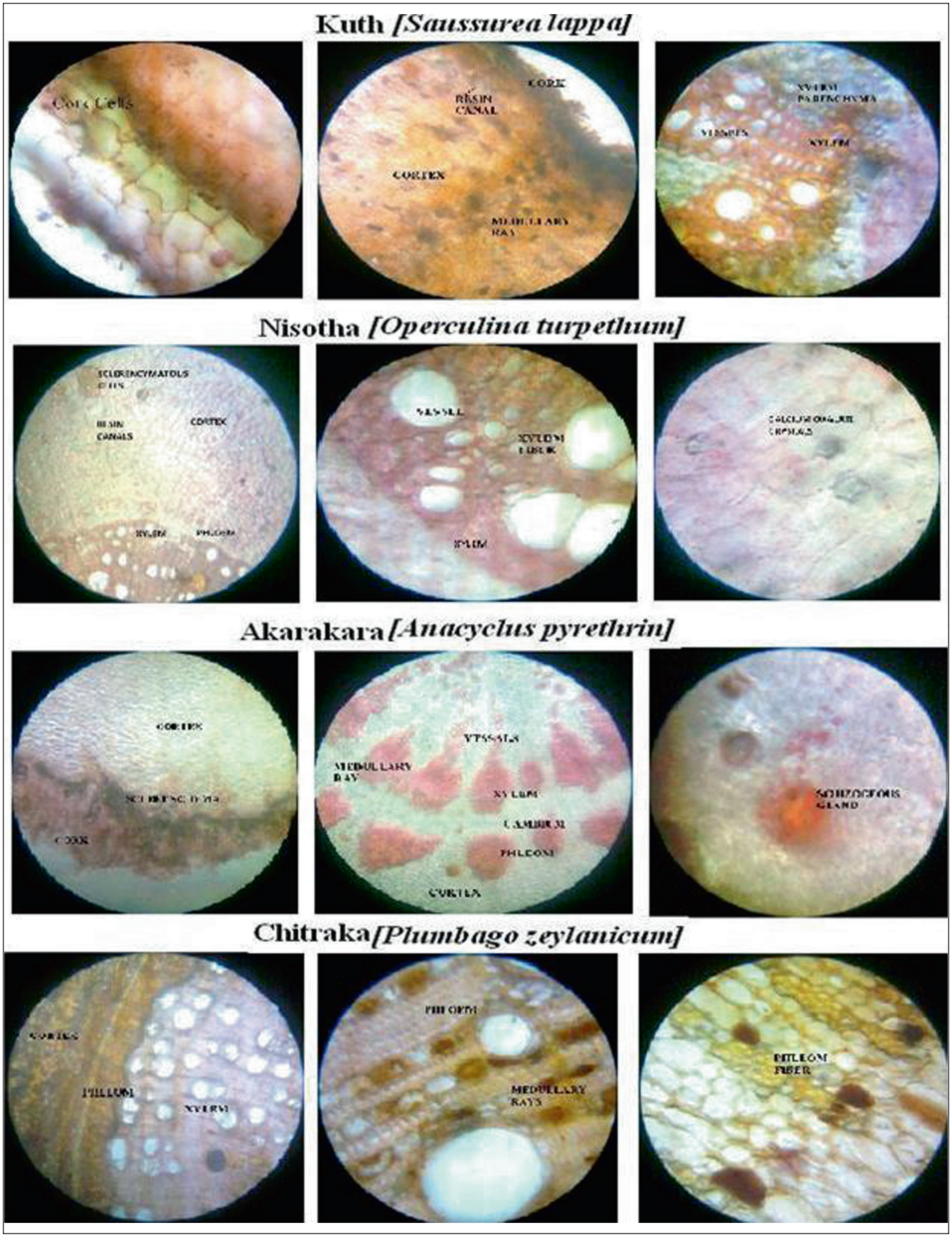

Figure 2: Microscopy of Kuth (Saussurea lappa), Nisotha (Operculina turpethum), Akarakara (Anacyclus pyrethrin), Chitrak (Plumbago zeylanicum)

Physicochemical analysis

Upon physicochemical analysis for all the roots samples on several parameters, outcome was matched with standard and presented in [Table 3][Table 4]. All the tests were performed in triplicate and result is presented in mean ± SEM.

Qualitative Phytochemical analysis

Upon phytochemical investigation of all the samples, different constituents were reported for all the samples. [Table 5]

Thin layer chromatography analysis

OT 1 showed five spots of violet color appearing at Rf. 0.20, 0.40, 0.49, 0.57 and 0.97. OT2 showed seven spots appearing at Rf 0.21, 0.41, 0.48, 0.58, 0.60, 0.62 and 0.96. OT3 showed seven spots appearing at Rf 0.21, 0.41, 0.49, 0.58, 0.60, 0.92 and 0.97. The reported numbers of spots are seven with Rf values 0.21, 0.41, 0.49 (all light violet), 0.58, 0.70, 0.90 and 0.97 (all violet) as per pharmacopoeia [Figure 3].

DISCUSSION

Kuth *(Saussurea lappa)*, Nisotha *(Operculina turpethum)*, Akarakara *(Anacyclus pyrethrin)* and Chitrak (*Plumbago zeylanicum)* are some of the very common plants used in Ayurvedic system of medicine. Evaluation of qualitative pharmacognostical parameters, physicochemical parameters and qualitative phytochemical screening can be useful in

Table 3: Results of extractive value analysis

	Water soluble extractive value	Standard value	Ethanol soluble extractive value	Standard value
SL1	14.73 ± 0.26%	Not less than 20 %	10.89 ± 0.12%	Not less than 12%
SL2	20.65 ± 0.32%		13.06 ± 0.29%	
SL3	18.87 ± 0.14%		12.78 ± 0.11%	
OT1	5.80 ± 0.52%	Not less than 8%	10.45 ± 0.26%	Not less than 10%
OT2	8.11 ± 0.17%		9.88 ± 0.56%	
OT3	9.86 ± 0.36%		10.67 ± 0.12%	
AP1	12.87 ± 0.08%	Not more than 22%	5.72 ± 0.12%	Not less than 8%
AP2	11.46 ± 0.65%		7.56 ± 0.32%	
AP3	15.73 ± 0.06%		9.40 ± 0.45%	
PZ1	13.47 ± 0.32%	Not less than 12%	16.10 ± 0.14%	Not less than 12%
PZ2	15.20 ± 0.22%		12.66 ± 0.24%	
PZ3	10.75 ± 0.18%		8.42 ± 0.33%	

Table 4: Results of ash value analysis

	Total Ash value	Standard value	Acid insoluble ash value	Standard value
SL1	5.23 ± 0.06%	Not more than 4%	2.82 ± 0.40%	Not more than 1%
SL2	3.61 ± 0.12%		0.97 ± 0.14%	
SL3	4.80 ± 0.31%		1.06 ± 0.23%	
OT1	12.58 ± 0.45%	Not more than 10%	2.13 ± 0.04%	Not more than 1.5%
OT2	11.36 ± 0.21%		2.53 ± 0.16%	
OT3	8.31 ± 0.19%		1.38 ± 0.23%	
AP1	8.43 ± 0.42%	Not more than 10%	2.05 ± 0.05%	Not more than 2%
AP2	10.54 ± 0.31%		1.94 ± 0.13%	
AP3	9.37 ± 0.07%		1.28 ± 0.06%	
PZ1	2.60 ± 0.14%	Not more than 3%	0.78 ± 0.12%	Not more than 1%
PZ2	1.96 ± 0.07%		0.54 ± 0.15%	
PZ3	3.81 ± 0.27%		1.30 ± 0.20%	

Table 5: Results of qualitative phytochemical analysis

	SL1	SL2	SL3	OT1	OT2	OT3	AP1	AP2	AP3	PZ1	PZ2	PZ3
Alkaloid	+	+	+	–	–	–	+	+	+	–	–	–
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	–	–	–	+	+	+
Tannins	–	–	–	–	–	–	–	–	–	–	–	–
Phenolic com.	+	+	+	–	–	–	–	–	–	–	–	–
Flavnoids	+	+	+	+	+	+	–	–	–	–	–	–
Fixed oil	–	–	–	–	–	–	–	–	–	–	–	–
Saponins	+	+	+	+	+	+	–	–	–	–	–	–
Proteins	–	–	–	–	–	–	–	–	–	–	–	–
Steroids	–	–	–	+	+	+	–	–	–	–	–	–

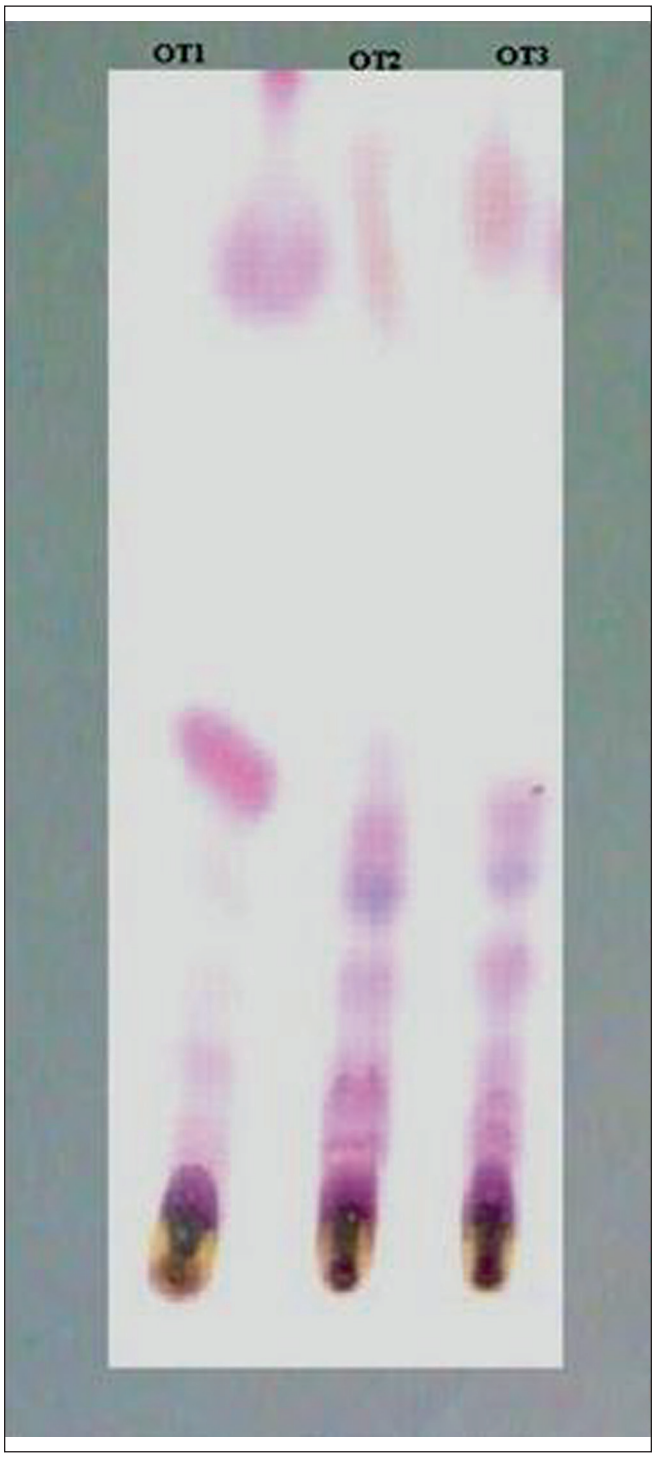

Figure 3: TLC Image of Nisotha (Operculina turpethum

standardization of the marketed samples of these drugs. In the present study, all samples were collected from different parts of Uttar Pradesh, India to evaluate the uniformity of the quality of raw materials used by small scale Ayurvedic drug manufacturers.

The samples were found almost uniform in there organoleptic properties. The variation observed may be due to the difference in storage conditions, collection process and age of plant. The qualitative pharmacognostical parameters were found uniform in all the samples.

The variation was observed in physicochemical properties; SL1, SL3, OT1, OT2, AP1, AP2 and PZ3 showed variation in all physicochemical parameters, from the standard value given in Ayurvedic Pharmacopoeia. The physicochemical parameters like extractive value, ash value indicates the quality and purity of drugs. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiologic ash and nonphysiologic ash. The variation in these parameters from standard value indicates the low quality of the samples .In qualitative Phytochemical analysis the sample were found uniform but in the thin layer chromatography results, absence of some spots in sample OT1 and OT2 was observed which indicates the absence of a particular group of compound in these samples.

CONCLUSION

By the above study, it can concluded that there is absence of uniformity in the quality of same plant material marketed in different area, which can result into variation in quality, safety and efficacy of same formulation manufactured in different area. Therefore emphasis is to be laid on the collection process, sources, storage conditions and standardization of raw materials in order to maintain the quality aspects of the product throughout worldwide**.**

ACKNOWLEDGEMENT

The authors are thankful to Managing Director, IFTM, Moradabad for providing all the laboratory facilities and chemicals to carry out this work.

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Pharmacognostic Evaluation and Phytochemical Studies on Stem of *Clitoria ternatea* linn.

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ABSTRACT

Pharmacognostic evaluation is the first and foremost step to determine identity and to assess the quality and purity of the crude drug. The genus *"Clitoria"* includes about 48 species under the family "Fabaceae". *Clitoria ternatea* a very common garden flowering plant is found all over India especially in southern India. *C. ternatea* have reported to possess a number of pharmacological activities such as nootropic, anxiolytic, anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic. The roots, stems and flowers are recommended for treatment of snake bite. The current study describes some pharmacognostical and preliminary phytochemical investigations undertaken on the stem of one of those species namely *Clitoria ternatea*. Transverse section of *Clitoria ternatea* Linn. stem shows cork, cortex, pericyclic fibers, xylem parenchyma, xylem fibers and medullary rays. Quantitative pharmacognostic analysis of the powder of the stem revealed moisture content of 5.92 ± 0.23%, total ash of 3.76 ± 0.32%, acid-insoluble ash of 11.6 ± 0.09%, alcohol extractive value of 9.84 ± 0.19%, and water extractive value of 24.8 ± 0.22%.

Key words: *Clitoria ternatea.*, Fabaceae , Pharmacognosy, Preliminary phytochemical screening

INTRODUCTION

Clitoria ternatea L. (Family: Fabaceae), commonly known as "Aparajita" is widely used as a substitute of shankhpushpi. It is a perennial twinning herb, steams are terete, more or less pubescent and leaves are imparipinnate, elliptic oblong, obtuse, glabrous or with a few short appressed hairs, flowers are axillary and solitary with pedicel.[1,2,3] There are two varieties of *Clitoria ternatea* white-flower and blue flower varieties. The leaves of both varieties contain an ester and resin glycosides. Seeds contain a fixed oil, a bitter acrid resin, tannic acid, glucose. Root bark contains starch, tannins and resins.[4,5] The roots have a sharp bitter taste and cooling, laxative, diuretic, anthelmintic, anti-inflammatory properties and useful in severe bronchitis, asthma, hectic fever and also used in ascites and abdominal enlargement (Ayurveda).[6] The infusion of root bark is used in gonorrhea and irritation of the bladder and urethra. The seeds are powerful cathartic, laxative and aperients actions in combinations with ginger powder. The roots, stems and flowers are recommended for treatment of snake bite.[7] The fatty acid content of *Clitoria ternatea* seeds includes palmitic, stearic, oleic, linoleic, and linolenic acids.[8,9,10] The seeds also contain a water-soluble mucilage, delphinidin 3, 3', 5'-triglucoside useful as a food dye; beta-sitosterol.[11,12] *C. ternatea* have number of pharmacological activities such as possessing nootropic, anxiolytic, antidepressant, anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic activities.[13,14,15] Hypoglycemic Activity of Methanolic Extract of *C.ternatea* Roots in Steptozotocin induced Diabetic Rat. Enhance memory, and increase acetylcholine content and acetylcholinesterase activity in rats.[16,17,18] Ethanol and benzene extract of *Clitoria ternatea* seeds at doses 75 mg/kg and 100 mg/kg inhibit clonidine induced catalepsy, milk induced eosinophili and leucocytosis in mice.[19,20]

The main objective of present study was to perform pharmacognostic investigation and preliminary phytochemical screening of the stem of *Clitoria ternatea* Linn.

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DOI: 10.5530/pj.2011.24.12

MATERIAL AND METHODS

Collection and Authentication

The stem of *Clitoria ternatea* L. was collected in bulk from local areas of Vallabh vidyanagar in August 2006, identified

and authenticated by taxonomist, Bioscience department, Sardar Patel University, Vallabh Vidyanagar. Remaining material was cut into small pieces and shade dried. The dried plant material was powdered using mechanical grinder, and sieved by using sieve No. 60. Then the final uniform powder was used for the extraction of active constituents of the plant.

Morphological and Microscopical studies

The morphology of the stem was studied according to standard methods. Transverse sections of the stem were taken manually, cleared, stained and mounted and representative photographs of sections were taken with the help of Digital microscope. The powder characteristics were studied according to standard methods.[21,22,23]

Determination of physicochemical parameters

Physico-chemical parameters i.e. percentage of moisture content, percentage of ash values and extractive values were performed according to the official methods.[23,24]

Preliminary phytochemical screening

The powdered plant was extracted with petroleum ether (60-80 °C), toluene, chloroform, acetone, methanol and distilled water using soxhlet apparatus. All the extracts were screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests.[23,24,25]

RESULTS

Morphological Evaluation

The stems were fleshy, long, slender and flexible with 0.5-3 m long, hairy or bald, sometimes somewhat erect stem. (Figure 1)

Fracture of the dried stem was fibrous and 15-20 cm in length, 5-10 mm in width.

Figure 1: Entire plant of *Clitorea ternatea*

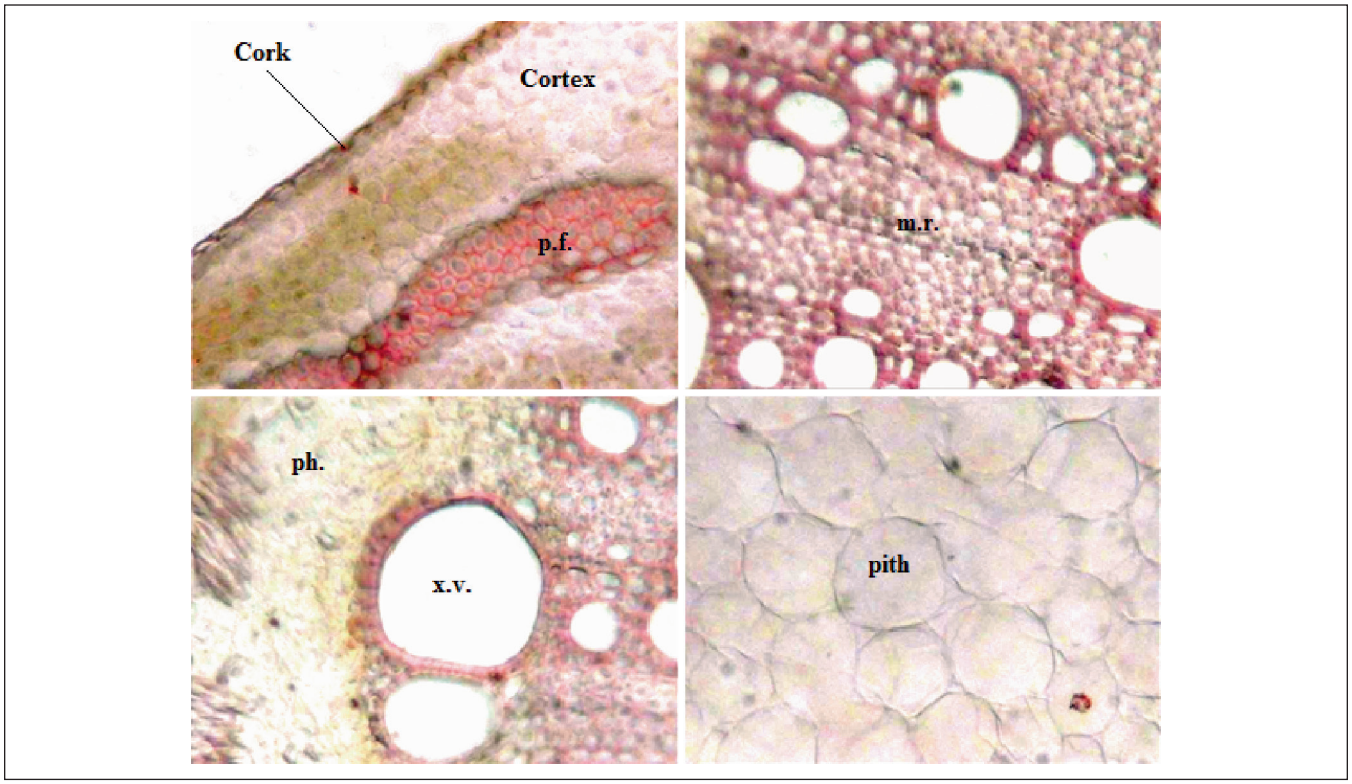

Figure 2: Transverse section of *Clitoria ternatea* stem
(p.f. = pericyclic fibres, m.r. = medullary rays, ph. = phloem, x.v. = xylem vessels)

Microscopical Evaluation

Transverse section of *Clitoria ternatea* Linn. stem (Figure 2) shows 1-2 layers of thin walled rectangular, tangentially elongated cork cells. The cortex followed by the cork was composed of 8-9 rows of thin walled parenchymatous cells, some of which contained chlorophyll. The cortex was followed by a continuous layer of lignified pericyclic fibre, the cells of which were polygonal, lignified and thick walled. The pericyclic fibres were followed by phloem which consisted of phloem parenchyma. Xylem as a continuous layer and wedge shaped was seen which consisted of large xylem vessels, xylem parenchyma and xylem fibres. Xylem parenchymatous cells were pitted and form the 1-5 serriate medullary rays. The centre of the stem was composed of the pith in which the cells were thin walled parenchymatous in nature. Starch and calcium oxalate were not observed.

Powder characters

The powder was green in colour and odour and taste are characteristic. Texture is fibrous.

Microscopical studies of the powder are shown in Figure 3 and contained xylem fibers (A), xylem vessels (B), cork (C), cork with cortex (D) and pericyclic fibers (E). Starch and crystals of calcium oxalate were not observed.

Physicochemical parameters

The proximate analysis result showed that the moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value were 5.92 ± 0.23%, 3.67 ± 0.32%, 0.12 ± 0.09%, 9.84 ± 0.19%, and 24.8 ± 0.22% respectively (Table 1). Successive solvent extractions were shown in percentage of yield along with physical appearance. The percentage yield values for petroleum ether, toluene, chloroform, acetone and methanol were 1.62%, 1.35%, 6.07%, 3.02%, and 6.01% respectively (Table 2). All extracts were then subjected to qualitative examination to detect the presence of phytoconstituents.

The preliminary phytochemical studies revealed that petroleum ether extract showed the presence of fixed oil and fats, toluene

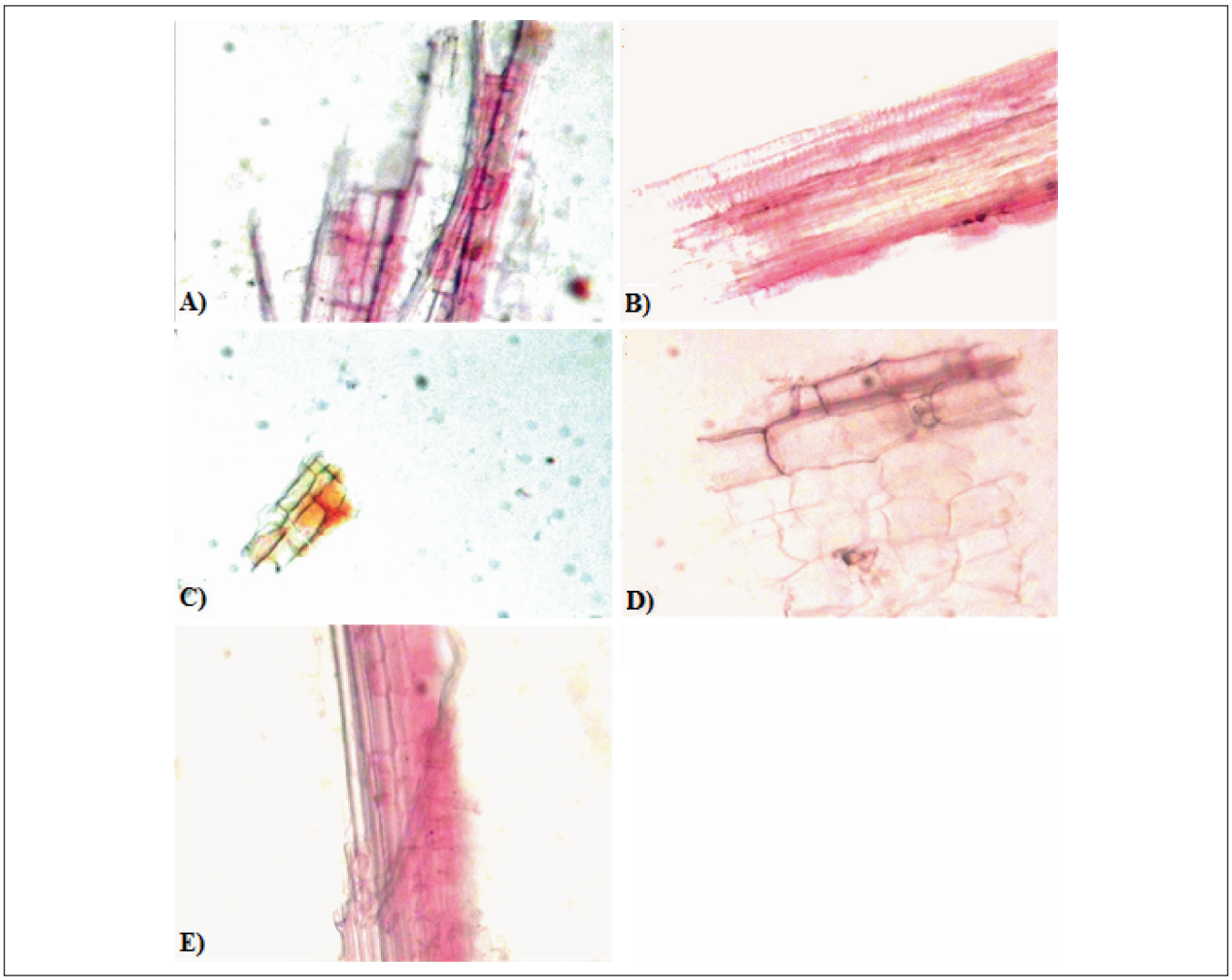

Figure 3: Powder characteristics of *Clitoria ternatea* stem

extract showed the presence of fixed oils, chloroform extract contained carbohydrates and saponins, acetone extract contained carbohydrates, saponins and phenolics, while methanolic extract contained carbohydrate, proteins and amino acids, saponin, phenolics (Table 3). The extracts which showed the presence of phytoconstituents of interest were further subjected to TLC and results are tabulated in Table 4.

Table 1: Evaluation of stems of *Clitoria ternatea* Linn.

Parameters	Value obtained on dry weight basis (% w/w)*
Moisture content	5.92 ± 0.23
Total ash value	3.67 ± 0.32
Acid insoluble ash	0.12 ± 0.09
Alcohol soluble extract	9.84 ± 0.19
Water soluble extract	24.8 ± 0.22

*Average of three reading ± SEM on of stems of *Clitoria ternatea* Linn.

Table 2: Successive solvent extraction of stems of *Clitoria ternatea* Linn.

Solvent used	Color & consistency	Average extractive values on dry weight basis (% w/w)
Petroleum ether	Green solid mass	1.62
Toluene	Dark green solid mass	1.35
Chloroform	Blackish green residue	6.07
Acetone	Dark Brown semisolid mass	3.02
Methanol	Reddish Brown solid mass	6.1

Table 3: Chemical examination of various extracts of stems of *Clitoria ternatea* Linn.

Constituents	Extract				
	P	T	C	A	M
Alkaloids	–	–	–	–	–
Carbohydrates	–	–	+	+	+
Proteins & amino acids	–	–	–	–	+
Saponin	–	–	+	+	+
Fixed oil/ Fat	+	+	–	–	–
Gums/ mucilage	–	–	–	–	–
Flavonoids	–	–	–	+	+
Phenolic	–	–	–	+	+

P = Petroleum ether extract; T = Toluene extract; C = Chloroform extract; A = Acetone extract; M = Methanol extract.

CONCLUSION

The various morphological, microscopical, physicochemical standards developed in this study will help for botanical identification and standardization of *Clitoria ternatea*. Further, the authentic plant material can be explored for its pharmacological and phytochemical potential.

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Table 4: TLC screening of various crude drug extract of *Clitoria ternatea* Linn.

Solvent system used	Detection Reagent	Observation	Inference	P	T	C	A	M
Toluene : Ethyl acetate (93:7)	VS reagent	Red/Yellow/Brown/Blue-green	Essential oil	+	+	–	–	–
	AS reagent	Pink/green	Essential oil	+	+	–	–	–
Ethyl acetate : Methanol : Water (100:13.5:10)	AS reagent	Red/Yellow/Brown/Blue-green	Bitter Principle	+	+	–	–	–
	VS reagent	Blue	Saponin	–	–	+	+	+
	NP/PEG/ and UV	Yellow/Green/Orange	Flavonoid	–	–	–	+	+
	10% KOH	Yellow colour	Anthrone	–	+	+	+	+
Chloroform : Methanol : Formic acid (10:0.3:0.1)	LB reagent	Dark green	Phytosterol	+	–	–	–	–

(P = Petroleum ether extract; T = Toluene extract; C = Chloroform extract; A = Acetone extract; M = Methanol extract, VS = Vanillin sulphuric acid, AS = Anisaldehyde sulphuric acid, LB = Libermann Burchard, + Indicates presence of constituents. – Indicates absence of constituents)

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Mast Cell Stabilization and Membrane Protection Activity of *Barleria prionitis* L.

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ABSTRACT

Barleria prionitis is a well-known medicinal plant, traditionally used for the treatment of various inflammatory diseases because of its anti-inflammatory activity. But the effect of the extract on inflammatory mediators and cell membrane in response to toxic chemicals was not studied before. Here, we evaluated the membrane stabilization and mast cell protection activity of hydroalcoholic extract of *B. prionitis* whole plant. The hydroalcoholic extract significantly inhibited the hypo-saline induced erythrocyte membrane hemolysis and the compound 48/80 induced mast cells degranulation in a dose dependent manner. The extract at dose concentration of 10 µg/ml, reduced the rat mesenteric mast cells degranulation up to 64.91% and prevented hypotonic solution induced hemolysis of rat erythrocytes by 27.10%. These findings clearly validate the anti-inflammatory activity of *B. prionitis* whole plant extract and provide support for traditional usage for inflammatory disorders.

Key words: *Barleria prionitis*, anti-inflammatory, phytochemical studies

INTRODUCTION

Barleria prionitis L. (Family: Acanthaceae) is a well-known medicinal plant traditionally used in Ayurveda for the treatment of bronchial asthma, rheumatic affections, inflammation, glandular swelling and this plant is found throughout the humid climatic zone of India.[1] The plant reported to contain barlerin, acetylbarlerin, scutellarein-7-neohesperidosideetc, balarenone, pipataline, lupeol, prionisides, barlerinoside, shanzhiside methyl ester, lupulinoside verbascoside, 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester and its cis-isomer.[1-3] The plant was reported to show antibacterial, anti-arthritic, anti-inflammatory,[4-5] hepatoprotective,[6] antioxidant,[7] antidiabetic,[8] anthelmintic,[9] antiviral,[2] and antifertility activities.[10] With this scope we have studied the erythrocytes membrane stabilization and mast cell protection activity of hydroalcoholic extract of *B. prionitis* whole plant against toxicants. Inflammation is a complex immunologic reaction frequently associated with pain which leads to increase of vascular permeability, protein denaturation and alteration of membrane integrity.[11] The mast cells plays important role in inflammatory events.[12] Hence, the present work has been highlighted the effect of hydroalcoholic extract of *B. prionitis* on inflammatory events such as membrane stabilization and mast cell protection.

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DOI: 10.5530/pj.2011.24.13

MATERIALS AND METHODS

Plant material

B. prionitis L. (Acanthaceae) whole plant was procured from local vendor and sample was authenticated from the Department of Botany and Forestry, Vidyasagar University, India. Voucher specimen (no: VBDB/10/17) was preserved in the Department of Botany and Forestry, Vidyasagar University, India.

Preparation of plant extract

The air dried plant material (300 g) was powdered in grinder and extracted with hydro-methanol (70%) by cold maceration process for 15 days to obtain crude extract. The crude extract was filtered through Whatman No. 1 filter paper and filtrate was evaporated and dried in a rotary evaporator followed by lyophilization. The percentage yield of the extract was calculated. The dried extract was dissolved in distilled water before use.

Animals

Male Wistar rats (200-225 g) were obtained from the authenticated vendor and the animal were housed in the animal facilities at Vidyasagar University. The animals were maintained in a laminar air-flow room at a temperature of 22 ± 1 ºC under the relative humidity of 55 ± 10% with standard food and water *ad libitum* throughout the study. The animal studies were done in accordance to the institutional guidelines and the protocols were approved by the Institutional ethical committee for Animal Care and Use at Vidyasagar University before study.

Reagents

Compound 48/80 (C 48/80) was purchased from Sigma-Aldrich (USA), O-toludine blue was obtained from Spectrochem Pvt. Ltd, India, disodium cromoglycate (DSCG) from Cipla, India. Indomethacin (INDO) was obtained from GenPharma International Pvt. Ltd. India. All other analytical grade solvents and reagents utilized in this study were purchased from the local vendors.

Phytochemical screening

The dried extract was subjected for the colour reactions to screen the nature of chemical components present in the extract. Small portion of dried extract was dissolved in purified water. The screening of phytochemical classes was performed according to the standard phytochemical procedures.[13-15]

Tests for alkaloids

The aqueous extract (2 ml) incubated with few drops of dilute hydrochloric acid in four different test tubes and then filtered. Each filtrate was tested with alkaloidal reagents either of the Mayer's (cream precipitate) or Dragendorffs (orange brown precipitate) or Hager's (yellow precipitate) or Wagner's (reddish-brown precipitate) reagents. Development of colour precipitates was observed in each test tube.

Tests for glycosides

The dried extract (50 mg) incubated with concentrated hydrochloric acid for 2 h in a water bath and filtered. Filtrate was subjected for Borntrager's test (pink colour) and Legal's test (pink colour). Development of pink colour in both tests indicated the presence of glycosides.

Tests for saponins

The dried extract (50 mg) dissolved in 20 ml of distilled water and shaken in graduated cylinder for 15 minutes. The formation of a 1-2 cm layer of foam indicated the presence of saponins.

Tests for flavonoids

The dried extract dissolved in distilled water and filtered. 5 ml of dilute ammonia solution was added with some portion filtrate followed by the addition of concentrated H_2SO_4. Formation yellow colouration indicated the presence of flavonoids and colouration disappeared on standing.

Tests for terpenoids

Salkowski test was performed to determine the presence of terpenoids. Five ml of aqueous extract was mixed with 2 ml of chloroform followed by the careful addition of 3 ml concentrated H_2SO_4 to form a layer. A reddish brown colour was formed in the inter face indicated the presence of terpenoids.

Test for tannins

The dried extract (50 mg) was dissolved in 20 ml of distilled water and filtered. With the filtrate few drops of 0.1% ferric chloride was added and the formation brownish-green or a blue-black colouration indicated the presence of tannins.

Test for steroids

The dried extract (50 mg) was dissolved in 2 ml of acetic anhydride. With this, 1-2 drops of concentrated H_2SO_4 was added slowly. The colour of reaction mixture was changed from violet to blue or green colour indicated the presence of phytosteroids.

Membrane stabilization activity

In vitro membrane stabilization was performed using the method of hypotonicity induced rat erythrocyte hemolysis described by Shinde and his co-workers (1999).[16] Whole blood of rats was collected by retro-orbital puncture using heparinized syringe. The whole blood samples were centrifuged at 3000 rpm for 10 minutes. The erythrocytes pellet was collected and washed by re-suspending with isotonic buffered solution (154 M NaCl in 10M phosphate buffer pH 7.4). The procedure repeated three times and each time erythrocytes were centrifuged at 3000 rpm for 10 minutes. The test sample consisted of varying concentrations of extract (10, 100 and 1000 µg/ml) or INDO (10 µg/ml) and 0.50 ml of stock erythrocyte suspension in 4.0 ml of hyposaline solution. The control sample consisted of 0.5 ml of stock erythrocyte suspension with hypotonic buffered saline solution. The reaction mixtures were incubated at 56 ± 1 °C for 30 min and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm. The inhibition percentage of erythrocyte haemolysis was calculated according to the method of Shinde et al (1999).[16]

$$\text{Inhibition of erythrocyte heamolysis (\%)} = \left[\frac{OD1 - OD2}{OD1}\right] \times 100$$

Where:
OD1 = Optical Density of control
OD2 = Optical Density of test samples

Mast cells protecting activity

The protection of mast degranulation induced C 48/80 was performed using the method describe by Norton (1954).[17] The overnight fasted male Wister rats were anesthetize with excess ether and cut the whole abdomen to expose the intestine. The intestinal mesenteries were collected in Ringer-Locke solution and the mesenteries were cut into small pieces. The pieces of mesentery were placed in different petri dishes consisting of different concentrations of extract (10, 100 and 1000 µg/ml) or DSCG (10 µg/ml) prepared in Ringer Locke solution. The petri-dishes were incubated with toxicant C 48/80 (0.8 µg/ml) at 37 ºC for 30 minutes. Two sets of control were prepared by incubating pieces mesentery with or without toxicant C 48/80 in Ringer-Locke solution. The tissues were placed on a clean microscopic slide and remove the fatty layer carefully. The trimmed tissues were stained with 4% formaldehyde solution containing 0.1% O-toludine blue for 30 minutes. The tissues were then de-stained by successive washing with acetone and xylene for 5 minutes. The stained mesentery pieces were examined under digital light microscope at 100X magnification. The mast cell was considered as degranulated if 4-5 granules were present around the cells. The percentage of degranulated and intact mast cells was calculated on the basis of counting 100 mast cells for each sample. For each set of test sample including control 6-8 pieces of mesentery were observed.

Statistical analysis

All the results were expressed as mean ± SEM of the number of the experiments. Statistical significance was performed by one-way ANOVA followed by Bonferroni's multiple comparison or Dunnett's multiple comparison test wherever applicable. The P values < 0.05 were considered as statistically significant. The data analysis was performed using Graph Pad Prism software.

RESULTS

Phytochemical screening

The vacuum-dried extracts gave 4.15% yield of whole plant extract. The results of preliminary phytochemical analysis of hydroalcoholic extract of whole plant are shown in Table-1. The extract gave positive results for the presence of glycosides, saponins, flavonoids, steriods and tannins.

Effect on protection of mast cells

The C 48/80 at dose concentration of 0.8 µg/ml significantly degranulated rat mesenteric mast cells (93.08 ± 4.59%, $P < 0.001$) in compared to the control (2.65 ± 0.84%). The hydroalcoholic extract showed dose dependent inhibition against C 48/80 induced mast cells degranulation as shown in Figure-1A and 1B. At a dose concentration of 10 µg/ml

Table 1. Phytochemical constituents of hydroalcoholic extract of *B. prionitis* whole plant

Test	Observation	Inference
Tests for alkaloids	No colour formation with Dragendorffs, Hager's, Mayer's, Wagner's alkaloidal reagents.	Absence of alkaloids
Tests for glycosides	Pink colour with Borntrager's and Legal's test	Presence of glycosides
Tests for saponins	Formation of foam upon shaking.	Presence of saponins
Tests for flavonoids	Yellow colouration up on the addition of dilute ammonia and concentrated H_2SO_4 & colouration disappeared on standing.	Presence of flavonoids
Tests for terpenoids (Salkowski test)	No reddish brown colour formation in inter face of $CHCl_3$ & H_2SO_4.	Absence of terpenoids
Test for tannins	Formation of blue-black colour with $FeCl_3$ solution.	Presence of tannin
Test for steroids	Change of violet to blue or green colour with acetic anhydride & H_2SO_4.	Presence of steroids

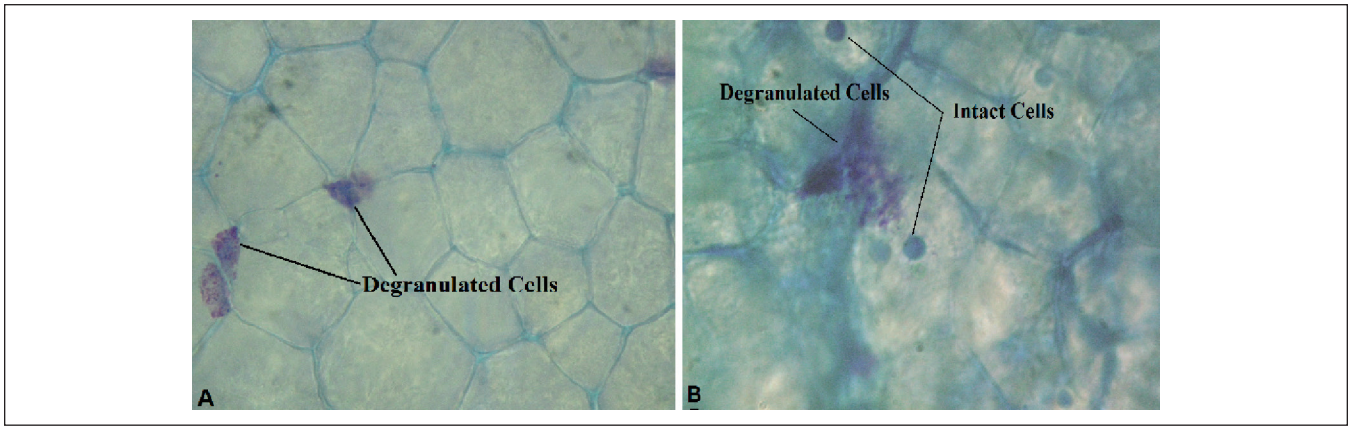

Figure 1: Mast cells protection by *B. prionitis* hydroalcoholic extract
A. Degranulated mast cells in control; B. Intact mast cells in hydroalcoholic extract treated sample.

extract reduced the degranulation of mast cells up to 64.91 ± 2.64% which was comparable with the standard DSCG (19.32 ± 6.92%, 10 µg/ml) with significance of $P < 0.001$, whereas 100 µg/ml of extract reduced the degranulation up to 31.42 ± 6.79% ($P < 0.05$) (Figure 2).

Effect on membrane stabilization

The extract at dose concentration of 10 µg/ml provided significant membrane protection (27.10 ± 3.18%) and the results were comparable with known standard drug, INDO (61.29 ± 6.37%, 10 µg/ml) with significance of $P < 0.01$, whereas 100 µg/ml extract showed membrane protection by 32.48 ± 5.26% ($P < 0.01$) (Figure 3).

DISCUSSION

The cell membrane integrity is essential for the normal growth, development and function of the cells. Exposure of erythrocytes to injurious medium, hyposaline solution, leads to rupture of its membrane followed by haemolysis and oxidation of haemoglobin.[18] The lysis of such bio-membranes leads to the generation of free radicals which enhanced the secondary cellular damage.[19] Thus, the compound with membrane-stabilizing property could offer significant protection of cellular membrane from toxic substances and interfere in early phase inflammatory reactions via inhibiting the formation of inflammatory mediators.[16]

The mast cells have an important role in the development of inflammatory anaphylactic and allergic reactions. During anaphylactic reaction IgE degranulated the mast cells to release histamine, heparin, proteases and other mediators to produce inflammatory effects.[20] These effects can be manipulated therapeutically by regulating the function of these mediators. Constituents from natural resources including plant origin may able to modulate such effects. In this experiment it was found that hydroalcoholic extract of *B. prionitis* whole plant provide significant mast cells stabilization and membrane protection.

CONCLUSSION

In conclusion, the result of the present investigation suggested that hydroalcoholic extract of *B. prionitis* whole plant has significant mast cell stabilizing and membrane protection activities. These effects validate the earlier reported anti-inflammatory activity and the traditional usage in inflammatory disorders. These effects could be the mechanism of action of anti-inflammatory action of this plant and this may due to the presence of glycosides, saponins, flavonoids, steroids, tannins in the extract. Thus, this plant may offer beneficial effects in the management of inflammatory conditions. However, further studies should be performed on isolated chemical compounds to establish the chemical responsible for the activity.

ACKNOWLEDGEMENTS

Authors are thankful to the Ulysses Research Foundation, India for providing financial assistance to carry out this research work.

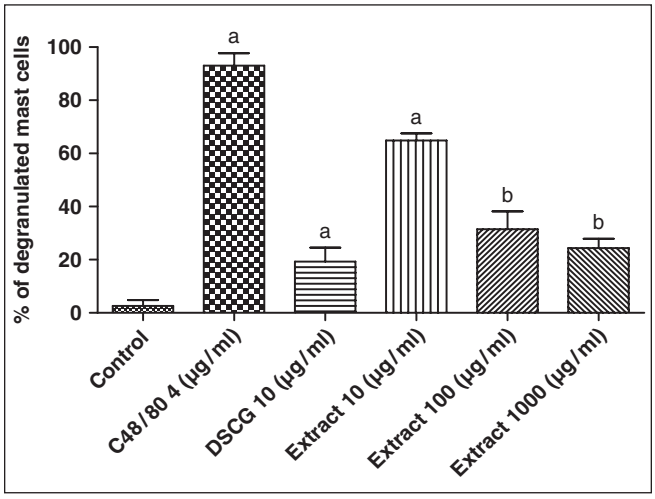

Figure 2: Mast cell protection activity of *B. prionitis* extract
The bars of the graph represents mean ± SEM of three observations (n = 3). Statistical analysis was done through One-way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison test. Control Vs C 48/80, a = P < 0.001; C 48/80 Vs DSCG (a = P < 0.001) and extract treated groups (a = P < 0.001; b = P < 0.01).

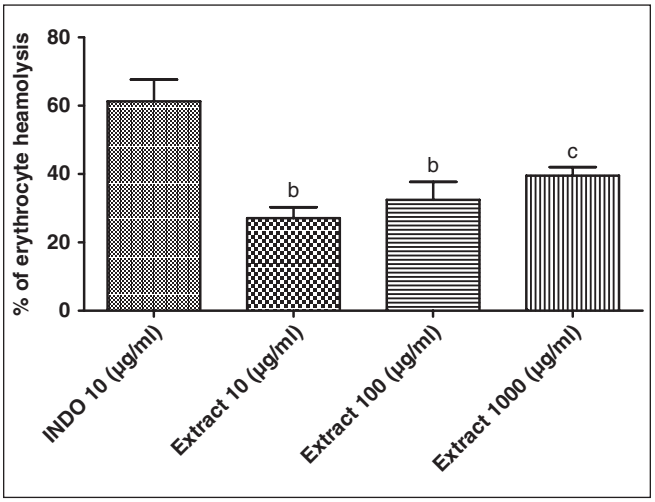

Figure 3: Membrane stabilization activity of *B. prionitis* extract
The bars of the graph represents mean ± SEM of three observations (n = 3). Statistical analysis was done through One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test. INDO Vs extract treated groups (b = P < 0.01; c = P < 0.05).

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In Vitro Antimicrobial Screening of Four Reputed Bangladeshi Medicinal Plants

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ABSTRACT

The antimicrobial activity of extractives of different plants has been recognized for many years. In present study the crude methanolic extracts and their kupchan partitioning fractions of four medicinal plants of Bangladesh namely, *Sansevieria trifasciata* (Fam: Asparagaceae), *Justicia gendarussa* (Fam: Acanthaceae), *Hydnocarpus kurzii* (Fam: Achariaceae) and *Kigelia pinnata* (Fam: Bignoniaceae) were investigated for their *in vitro* antimicrobial properties. All fractions were tested against 11 different gram positive and gram negative bacteria by the disc diffusion technique for bacteria, where kanamycin (30 µg/disc) disk used as standard. Among the extractives, the methanol extract and their pet-ether, carbon tetrachloride and chloroform soluble kupchan fractions of leaf extract of *H. kurzii*, and the aerial part extract of *S. trifasciata* showed significant antibacterial activity, where as chloroform soluble extracts of leaves of *H. kurzii* revealed highest activity against *Vibrio mimicus* (15.00 mm) and the methnol extract of whole plant of *S. trifasciata* demonstrated highest activity against *V. mimicus* (14.67 mm). The chloroform soluble fractions of *J. gendarussa* also showed mild to moderate antimicrobial activity with zone of inhibition ranging from 8.33-13.00 mm, in which highest activity was seen against *Shigella boydii* (13.00 mm). All the extractives of *K. pinnata,* on the other hand, demonstrated mild antimicrobial activity, where highest zone of inhibition was displayed by the chloroform soluble extract against *S. boydii* (11.00 mm) and *Pseudomonas aeruginosa* (11.00 mm).

Key words: Antimicrobial activity, Disc diffusion, *Hydnocarpus kurzii, Justicia gendarussa, Kigelia pinnata* and *Sansevieria trifasciata*.

INTRODUCTION

The shade-loving, quick-growing, evergreen plant *Justicia gendarussa* (Burm F.) (Family: Acanthaceae), is mostly found in moist areas. It is believed to be native to China and is distributed widely across India, Sri Lanka, and Malaysia.[1] It is an erect, branched, smooth undershrub, 0.8-1.5 meters in height with long leaves (7 to 14 cm).[2] The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever. The plants of this genous are known to contain lignans, naturally occurring phenolic dimers and triterpenoids.[3] *Sansevieria trifasciata* (Prain) (Family: Ruscaceae), commonly known as snake plant or mother in-law's tongue is an evergreen herbaceous perennial plant which is found throughout Malaysia[4] where it has been traditionally used for the treatment of ear pain, swellings, boils and fever. Phytochemical screening with this plant has shown to contain carbohydrates, saponins, glycosides[5] and steroids.[6]

Hydnocarpus kurzii (King Warb) (Family: Achariaceae also placed in: Flacourtiaceae[7-8]) is well known for its chaulmoogra oil, which is expressed from the dried ripe seeds[9] and is also known as hydnocarpus oil, kalaw tree oil, leprosy oil.[10] The oil and the crushed seeds have long been used in southeast Asia to treat various skin diseases like scabies, eczema, psoriasis, scrofula, ringworm, and intestinal worms and it has been shown that the active principles of the oil (hydnocarpic and chaulmoogric acids) exhibited strong antibacterial activity. For this reason *H. kurzii* is employed in Hindu medicine to treat leprosy. The bark contains principles capable of reducing fevers. Seeds are usually applied externally as a dressing for skin diseases; combined with walnut oil and pork lard for ringworm; with calomel

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DOI: 10.5530/pj.2011.24.14

and sesame oil for leprosy; and with sulfur and camphor for scabies. In India, the seeds are considered to be an alternative tonic.[11]

Kigelia pinnata (Lam. Benth) belonging to the family of Bignoniaceae and has a wide geographical distribution in the west and central Africa. The tree grows on river banks, wet areas along streams and on floodplains of Nigeria, Cameroon, Kenya, Guinea and Senegal.[12] The kigelia plant have medicinal properties not only because of its perceived characteristics such as bitterness, astringent taste or smell but also because of forces that it seems to emit in connection with its location, orientation and association with other plants.[13] *K. pinnata* is widely used for antidiarrhoeal,[14] antileprotic,[15] antimalarial,[16] anti-inflammatory,[12] anticancer,[17] gynecological disorders,[18] anti-microbial[19] and rheumatism.[20]

We, here in, report the results of preliminary antimicrobial screening of the methanolic crude extracts and the corresponding Kupchan partitioning fractions of four Bangladeshi medicinal plants having folklore reputation for the first time.

MATERIAL AND METHODS

Collection and Preparation of the Plant Materials

J. gendarussa, *S. trifasciata*, and *H. kurzii* were collected from Dhaka Botanical garden in February 2011 while *K. pinnata* was obtained from Rangpur in February 2010, voucher specimens (DACB 35489, 35490, 35491 and 34998, respectively) have been deposited in Bangladesh National Herbarium for future reference. Leaves and bark of *J. gendarussa*, whole plant of *S. trifasciata* and leaves of *H. kurzii* and *K. pinnaata* were sun dried for several days after washing. The plant materials were then oven dried for 24 hours at 40 °C and then ground to a coarse powder. The powdered materials (300 gm each) were then soaked in methanol (1.5 liter each) and kept for 10 days at room temperature with occasional shaking. The crude extracts were then filtered through cotton plug followed by Whatman no. 1 filter paper individually and the extracts were concentrated with rotary evaporator.

Extraction and Fractionation

A portion (5 g) of each of the concentrated methanol extract (ME) was fractionated by the modified Kupchan partitioning method[21] into pet-ether (PESF), carbon tetrachloride (CTSF), chloroform (CFSF), and aqueous (AQ) soluble fractions. Evaporation of solvents afforded various organic soluble extractives and aqueous soluble materials as shown in Table 1.

Test Organisms

Both gram positive (*Bacillus cereus, B. subtilis, Sarcina lutea, Staphylococcus aureus*) and gram negative (*Escherichia coli, Salmonella paratyphi, Shigella boydii, S. dysenteriae, Pseudomonas aeruginosa, Vibrio mimicus, V. parahemolyticus*), bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Table 1: Amount of different partitionates of *J. gendarussa, S. trifasciata, H. kurzii, K. pinnaata*

Partitionates	*J. gendarussa*	*S. trifasciata*	*H. kurzii*	*K. pinnaata*
pet-ether (PESF)	730 mg	750 mg	720 mg	720 mg
carbon tetrachloride (CTSF)	500 mg	520 mg	550 mg	600 mg
chloroform (CFSF)	480 mg	470 mg	410 mg	580 mg
aqueous (AQ) soluble fractions	1200 mg	1100 mg	1350 mg	1420 mg

Table 2: Antimicrobial activity of crude extracts and Kupchan fractions of *Sansevieria trifasciata*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTSF	CFSF	AQ	Kanamycin
Gram positive bacteria						
Bacillus cereus	10.00 ± 1.00	9.33 ± 2.31	12.67 ± 1.15	14.00 ± 1.00	–	32.00 ± 1.00
B. subtilis	12.67 ± 2.08	10.33 ± 1.53	14.00 ± 1.00	13.67 ± 1.53	–	34.00 ± 1.00
Staphylococcus aureus	10.67 ± 1.15	11.67 ± 0.58	11.67 ± 2.08	11.67 ± 0.58	–	34.33 ± 1.15
Sarcina lutea	11.33 ± 2.08	12.00 ± 2.00	10.00 ± 1.00	12.33 ± 2.08	–	33.67 ± 2.31
Gram negative bacteria						
Escherichia coli	11.33 ± 1.53	11.00 ± 2.00	13.67 ± 1.53	12.67 ± 1.15	8.33 ± 1.53	32.67 ± 1.15
Pseudomonas aeruginosa	12.00 ± 3.00	11.67 ± 0.58	11.33 ± 1.15	9.00 ± 1.00	–	33.00 ± 1.00
Salmonella paratyphi	11.67 ± 2.08	11.33 ± 2.31	12.33 ± 1.53	11.67 ± 1.53	–	32.00 ± 2.65
Shigella boydii	12.33 ± 2.52	11.67 ± 2.08	12.00 ± 2.00	12.00 ± 2.65	–	33.67 ± 0.58
S. dysenteriae	11.67 ± 1.15	10.33 ± 0.58	10.33 ± 0.58	11.67 ± 2.08	–	32.67 ± 2.52
Vibrio mimicus	14.67 ± 2.00	11.67 ± 1.53	13.67 ± 1.53	13.33 ± 1.15	–	33.33 ± 2.08
Vibrio parahemolyticus	12.00 ± 2.00	12.00 ± 1.73	12.67 ± 2.08	12.67 ± 1.15	–	33.00 ± 1.00

Experimental Procedure

The antimicrobial study was carried out by disc diffusion technique for bacteria.[22] Standard kanamycin disc (Kan.) (30 µg/disc) and discs containing the test materials (400 µg/disc) and respective solvents were used as positive and negative controls, respectively. According to this method, the antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

RESULTS AND DISCUSSION

In our preliminary antimicrobial screening of four local medicinal plants, it was observed that, among different fractions the methanol extract and its pet-ether, carbon tetrachloride, chloroform soluble fractions of leaves of *H. kurzii* showed significant activity against most of the gram positive and gram negative bacteria at a dose of 400 µg/disc (Tables 2-5). All the extractives of *S. trifasciata* exhibited moderate antimicrobial activity, (Table 2) especially the methnol extract revealed mild to moderate antimicrobial activity with zone of inhibition ranging from 10.00-14.67 mm with the highest being seen against *Vibrio mimicus* (14.67 mm). Moderate activity was seen against *Bacillus subtilis* (12.67 mm), *Vibrio parahemolyticus* and *Pseudomonas aeruginosa* (12.00 mm each). The chloroform soluble fraction of this palnt also revealed mild to moderate antimicrobial activity (9.00-14.00 mm), where highest activity was found against *Bacillus cereus* (14.00 mm). Different extractives of leaves of *J. gendarussa* also demonstrated mild to moderate antimicrobial activity and its chloroform soluble Kupchan partitionate showed antimicrobial activity with zone of inhibition (Table 3) ranging from 8.33-13.00 mm. This fraction revealed strong activity against *B. cereus* (13.33 mm) and moderate inhibitory activity agent *Salmonella paratyphi* (12.67 mm). On the other hand, the chloroform soluble partitionate of the methanol extract of leaves of *H. kurzii* exerted significant antimicrobial activity having inhibitory zone (Table 4) ranging from 9.00-16.00 mm. The chloroform soluble fraction demonstrated antimicrobial activity against gram positive bacteria like *B. cereus* (11.67 mm) *B. subtilis* (11.33 mm), *Sarcina lutea* (11.33 mm) and gram negative bacteria like *Escherichia coli* (11.67 mm), *P. aeruginosa* (14.00 mm), *S. paratyphi* (14.00 mm),

Table 3: Antimicrobial activity of crude extracts and Kupchan fractions of *Justicia gendarussa*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTSF	CFSF	AQ	Kanamycin
Gram positive bacteria						
Bacillus cereus	11.67 ± 1.53	11.33 ± 0.58	–	13.33 ± 1.53	–	32.33 ± 1.53
B. subtilis	9.00 ± 1.00	11.67 ± 2.08	–	8.33 ± 1.53	–	34.00 ± 1.00
Staphylococcus aureus	9.33 ± 0.58	11.00 ± 1.00	–	11.33 ± 2.08	–	32.00 ± 1.00
Sarcina lutea	9.67 ± 1.53	8.67 ± 1.15	–	8.33 ± 1.53	12.33 ± 2.52	33.33 ± 1.53
Gram negative bacteria						
Escherichia coli	10.00 ± 1.00	11.00 ± 1.00	–	12.33 ± 0.58	–	34.33 ± 1.53
Pseudomonas aeruginosa	10.33 ± 0.58	8.33 ± 0.58	–	8.33 ± 1.53	–	33.67 ± 2.31
Salmonella paratyphi	10.00 ± 1.00	11.00 ± 1.73	–	12.67 ± 1.15	–	32.67 ± 2.08
Shigella boydii	11.00 ± 1.73	13.00 ± 1.73	–	11.67 ± 0.58	–	32.00 ± 1.00
S. dysenteriae	12.00 ± 1.00	10.00 ± 1.00	–	12.33 ± 2.08	–	33.67 ± 1.15
Vibrio mimicus	9.67 ± 0.58	11.33 ± 1.15	–	11.00 ± 1.73	–	33.00 ± 2.00
Vibrio parahemolyticus	11.00 ± 2.65	11.67 ± 2.00	–	11.67 ± 0.58	–	31.67 ± 0.58

Table 4: Antimicrobial activity of crude extracts and Kupchan fractions of *Hydnocarpus kurzii*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTSF	CFSF	AQ	Kanamycin
Gram positive bacteria						
Bacillus cereus	12.67 ± 1.53	9.00 ± 1.00	11.67 ± 1.53	11.67 ± 1.15	–	31.67 ± 0.58
B. subtilis	11.00 ± 1.00	9.67 ± 0.58	10.33 ± 0.58	11.33 ± 1.53	–	32.33 ± 1.53
Staphylococcus aureus	14.67 ± 0.58	10.67 ± 0.58	11.67 ± 2.08	10.33 ± 1.15	–	33.67 ± 1.15
Sarcina lutea	14.67 ± 1.15	12.33 ± 0.58	11.33 ± 1.53	11.33 ± 1.15	–	32.00 ± 2.65
Gram negative bacteria						
Escherichia coli	11.33 ± 1.53	11.33 ± 1.53	12.67 ± 1.15	11.67 ± 1.53	12.00 ± 2.00	32.00 ± 1.00
Pseudomonas aeruginosa	10.67 ± 0.58	10.33 ± 0.58	10.33 ± 1.53	14.00 ± 1.00	–	32.67 ± 0.58
Salmonella paratyphi	12.67 ± 0.58	11.00 ± 1.73	9.67 ± 1.15	14.00 ± 2.00	–	32.67 ± 2.08
Shigella boydii	10.33 ± 1.15	11.00 ± 1.00	12.00 ± 1.00	11.67 ± 1.53	–	31.67 ± 0.58
S. dysenteriae	10.67 ± 0.58	12.00 ± 2.00	10.67 ± 2.08	11.67 ± 0.58	–	33.67 ± 1.15
Vibrio mimicus	10.33 ± 0.58	11.67 ± 0.58	11.00 ± 1.00	15.00 ± 1.00	–	31.67 ± 2.08
Vibrio parahemolyticus	11.00 ± 1.00	9.67 ± 0.58	11.33 ± 1.15	12.33 ± 1.53	–	30.67 ± 0.58

Table 5: Antimicrobial activity of crude extracts and Kupchan fractions of *Kigelia pinnaata*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PESF	CTSF	CFSF	AQ	Kanamycin
Gram positive bacteria						
Bacillus cereus	9.67 ± 1.53	10.33 ± 1.53	7.67 ± 0.58	8.33 ± 0.58	–	30.67 ± 0.58
B. subtilis	8.00 ± 1.73	8.67 ± 2.08	9.00 ± 1.53	9.67 ± 1.53	–	31.67 ± 1.53
Staphylococcus aureus	7.67 ± 1.15	10.33 ± 0.58	9.33 ± 0.58	9.67 ± 0.58	–	33.00 ± 1.73
Sarcina lutea	7.33 ± 0.58	9.00 ± 1.00	10.67 ± 1.53	10.33 ± 1.53	–	33.67 ± 1.15
Gram negative bacteria						
Escherichia coli	8.00 ± 1.00	7.33 ± 0.58	8.67 ± 1.53	9.33 ± 1.53	–	32.67 ± 2.08
Pseudomonas aeruginosa	9.67 ± 1.15	9.33 ± 0.58	8.33 ± 1.00	11.00 ± 1.00	–	33.00 ± 1.00
Salmonella paratyphi	7.33 ± 0.58	10.33 ± 1.53	8.33 ± 2.00	10.00 ± 2.00	–	33.33 ± 2.08
Shigella boydii	9.33 ± 1.53	8.67 ± 1.15	9.00 ± 1.00	11.00 ± 1.00	–	32.00 ± 1.00
S. dysenteriae	7.33 ± 1.15	8.33 ± 1.53	9.00 ± 1.53	8.33 ± 1.53	–	33.33 ± 0.58
Vibrio mimicus	8.00 ± 1.00	9.33 ± 2.52	9.00 ± 2.08	9.67 ± 2.08	–	32.00 ± 1.00
Vibrio parahemolyticus	8.67 ± 1.53	10.33 ± 0.58	8.00 ± 1.00	10.00 ± 1.00	–	34.00 ± 1.73

The average values of three calculations are presented as mean ± S.D. (standard deviation); ME = Methanolic extract; PESF = Pet-ether soluble fraction; CTSF = Carbon tetrachloride soluble fraction; CFSF= Chloroform soluble fraction; AQ = Aqueous soluble fraction of the methanolic extract of the plants.

V. mimicus (15.00 mm) and *V. parahemolyticus* (12.33 mm). Among the extractives of leaves of *K. pinnaata*, the chloroform soluble extractive showed weak antimicrobial activity (8-11 mm zone of inhibition) with better antimicrobial activity against gram negative bacteria, especially *P. aeruginosa* and *Shigella boydii* (11.00 mm each) (Table 5).

CONCLUSION

The methanolic extracts of leaf of *S. trifasciata, J. gendarussa, H. kurzii* and *K. pinnata* and their kupchan fractions (pet-ether, carbon tetrachloride and chloroform) demonstrated mild to moderate antibacterial activity against gram +ve and gram –ve bacteria.

The antibacterial activity demonstrated by the extractives of *S. trifasciata, J. gendarussa, H. kurzii* and *K. pinnata* are in agreement with the folk uses of the plants against chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever. However, further studies are warranted to isolate and characterize the compounds reported for the antimicrobial property.

ACKNOWLEDGEMENT

We are grateful to the department of Pharmacy, State University of Bangladesh, for materialistic supports and to the Institute of Food and Nutrition, University of Dhaka, for supplying the pure standard microorganisms.

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Evaluation of *in vitro* anthelminthic activity of *Leucas aspera* extracts

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ABSTRACT

Helminths infections are also among the most common infections in human, affecting a large proportion of the world's population in developing countries and produce a global burden of disease. *Pherithema posthuma* a helminthes is commonly known as earth-worms, *Leucas aspera* herb is distributed throughout India. The anthelminthic property of *Leucas aspera* was evaluated using *Pherithema posthuma* as an experimental model. Piperazine citrate was used as the standard reference. Earthworm belonging to control group showed paralysis time as 64.33 min and death time as 200 min. Among the various concentrations of aqueous extract tested, concenration at 250 mg/ml showed efficient anthelminthic activity and among all the concentrations ethanol extract tested, concentration at 250 mg/ml gave significant results. This investigation revealed that ethanol extract of *Leucas aspera* showed significant anthelminthic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Leucas aspera* as anthelminthic plant.

Key words: Lamiaceae; *Leucas aspera*; Anthelminthic activity; *Pheretima posthuma*; Ethanol extract; Aqueous extract.

INTRODUCTION

Parasitic helminthes are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts' nutrient absorption, causing weakness and disease in human and animals inflicting heavy production losses. Helminths infections are also among the most common infections in human, affecting a large proportion of the world's population in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia which more often physically impair their hosts than kill them.[1] Anthelminthics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them.[2] Various problems have been evolved with chemotherapeutic control practices such as parasites are developing resistance to several families of chemical anthelminthics,[3] chemical residues and toxicity problems,[4] un-economical and nonavailability of drugs in remote areas. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health.[5] For these various reasons, interest in the screening of medicinal plants for their anthelminthic activity remains of great scientific significance despite extensive use of synthetic chemicals in modern clinical practices all over the world.[6]

Helminthes infections are commonly found in community and being recognized as cause of much acute as well as chronic illness among the various human beings as well as cattle's. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections.[7] However, the high cost of modern anthelminthics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelminthics[8] has led to development of resistance and hence a reduction in the usefulness of available anthelminthics.[9] Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of

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DOI: 10.5530/pj.2011.24.15

helminth parasites, and as a means of reducing the cost of controlling helminthic diseases.[10-13]

Leucas aspera (Willd.) Linn. (Family: Lamiaceae), a herb commonly known as 'Thumbai is distributed throughout India from the Himalayas down to Ceylon.[14] The plant is used as an insecticide and indicated in traditional medicine for coughs, colds, painful swellings, and chronic skin eruptions.[15] Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.[14,16] Compounds isolated from the plant include, long-chain aliphatic compounds, a triterpene-leucolactone, sterols- sitosterol, campesterol, stigmasterol and a novel phenolic compound.[17-20] However, anthelminthic activity of *Leucas aspera* whole plant extract is not scientifically and reported. To justify the traditional claims of *Leucas aspera,* we made an efficient attempt to assess the anthelminthic activity of *Leucas aspera.*

MATERIALS AND METHODS

Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China.

Plant Resource

Leucas aspera plant material was collected from agricultural fields of Tinsukia, Assam, India. The plant was authenticated by Prof. V. Krishna, Kuvempu University. Fresh plant material was washed thoroughly in tap water to remove traces of soil and other contaminants. It is then shade dried. Further, the whole plant was chopped finely and was shade dried, powdered mechanically. The powdered plant material was transported in a vacuum sealed container to Bangalore for further experimental studies. This plant material was subjected to cold extraction using ethanol as the solvent system for about 96 h, after every 24 h fresh ethanol was added and ethanol containing the extract was separated, followed by double distilled water (with 5% ethanol, to avoid microorganism contamination) for 96 h. Both the extracts were filtered and concentrated in vacuum under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of ethanol crude and aqueous extract for 1 kg of powdered plant material was 36 g and 45 g respectively.

Test organism

Indian adult earthworms (*Pheretima posthuma)* collected from the Indo-American Hybrid Seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water, for about two weeks. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected model for anthelminthic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.[21,22]

Extract preparation for experiment

The porously powdered plant material was used for extract preparation. After extraction, the crude extract was stored in dessicator until further use. Ethanol extract and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v). Whereas, the crude aqueous extract was directly dissolved in normal saline and used for evaluation for anthelminthic activity.

Antihelminthic activity

The anthelminthic activity of whole plant extracts of *Leucas aspera* was evaluated as per the method reported by Dash et al.[23] Twelve groups of animals with three earthworms in each groups, each earthworm were separate released into 20 ml of desired formulation in normal saline, Group 1 earthworm were released in 20 ml normal saline in a clean petri plate. Group II, III, IV, V, VI earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of ethanol extract respectively. Similarly, group VII, VIII, IX, X, XI earthworms were released in 20 ml normal saline containing 50, 100, 150, 200 and 250 mg/ml of aqueous extract respectively. Group XII earthworms were released in 20 ml normal saline containing standard drug piperazine citrate (50 mg/ml). Earthworms were observed; the time taken for paralysis and the time taken for death was monitored and documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total lose of motility with faded body color.[24] The result of anthelminthic activity is depicted in Table 1.

Statistical analysis

The data of anthelminthic evaluations were expressed as mean ± S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P < 0.01$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

RESULTS AND DISCUSSION

Leucas aspera is a well known medicinal plant and is widely used in folk medicine/ ayurvedic system of medicine. In

Table 1: *In vitro* anthelminthic activity of ethanol and aqueous extracts of *Leucas aspera* against *Pheretima posthuma*

Test samples	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Normal Saline)		64.33 ± 0.88	200.00 ± 2.60
Ethanol extract of *Vinca rosea*	50	20.00 ± 0.58**	26.33 ± 0.88**
	100	16.00 ± 0.58**	23.00 ± 0.58**
	150	10.00 ± 0.33**	14.00 ± 0.58**
	200	6.33 ± 0.33**	11.00 ± 0.58**
	250	4.33 ± 0.88**	9.33 ± 0.33**
Aqueous extract of *Vinca rosea*	50	117.00 ± 1.15**	168.33 ± 4.06**
	100	100.67 ± 1.45**	154.00 ± 8.14**
	150	62.00 ± 1.53^{NS}	123.33 ± 2.60**
	200	56.00 ± 0.58**	115.00 ± 2.65**
	250	50.33 ± 0.88**	96.67 ± 2.33**
Piperazine citrate	50	31.33 ± 1.86**	40.67 ± 0.88**

Values are the mean ± S.E.M. of three earthworms. Symbols represent statistical significance. * $P < 0.05$, ** $P < 0.01$, ns: not significant as compared to compared to control group.

the present study solvents namely ethanol and water were used sequentially for crude extraction of *Leucas aspera* whole plant. To justify the ethnomedical claims of *Leucas aspera* we made an efficient attempt in evaluating the anthelminthic property of *Leucas aspera.*

Earthworm belonging to control group showed paralysis time as 64.33 min and death time as 200 min. Aqueous extract at the concentration of 50 mg/ml showed the time of paralysis and death at 117 and 168 min respectively. For concentration of 100 mg/ml, the paralysis and the death time was found to be 100.67 and 154 min respectively. At the concentration of 150, 200 and 250 mg/ml, time taken to paralysis was 62, 56 and 50.33 min respectively and death time 123.33, 115 and 96.67 min respectively. Among the various concentrations tested, aqueous extract at 250 mg/ml showed efficient anthelminthic activity (Table 1). On the other hand ethanol extract at the concentration of 50 mg/ml showed the time of paralysis and death at 20 and 26.33 min respectively. For concentrations at 100, 150, 200 and 250 mg/ml paralysis was shown at 16, 10.67, 6.33 and 4.33 min respectively and death occurred at 23, 14, 11 and 9.33 min respectively. Among all the concentrations ethanol extract tested, concentration at 250 mg/ml gave significant results. Standard drug at 50 mg/ml showed paralysis at 31.33 min and death time was 40.67 min (Table 1). This investigation revealed that ethanol extract of *Leucas aspera* showed significant anthelminthic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *Leucas aspera* as anthelminthic plant.

ACKNOWLEDGEMENT

The authors are grateful to Department of Biotechnology, The Oxford College of Science, Bangalore, for providing the facilities to carry out the entire experiment.

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Evaluation of Laxative Activity of *Oxystelma esculentum*

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ABSTRACT

Oxystelma esculentum is a perennial twiner growing near water-logged areas in the Indian subcontinent. It is used traditionally as a laxative. The present work deals with the investigation of laxative potential of various extracts of *O. esculentum.* The plant was successively extracted with solvents of varying polarities, which served as the test extracts. Laxative effect was checked in Wistar rats using different models. The petroleum ether extract was found to possess the most effective laxative activity, thereby supporting the traditional claim of the plant as a laxative. Phytochemical screening of this extract revealed the presence of important classes of compounds like cardenolides, flavonoids, phenolics, sterols and triterpenoids. This bioactivity-guided phytochemical screening can guide further therapeutic investigations and isolation of therapeutically important compounds from *Oxystelma esculentum*.

Key words: Laxative, *Oxystelma esculentum*, *Oxystelma secamone*, *Periploca esculenta*

INTRODUCTION

Oxystelma esculentum R. Br. syn. *Oxystelma secamone* Linn., *Periploca esculenta* Roxb., *Periploca secamone* Linn., *Sarcostemma secamone* Bennet, *Sarcostemma esculentum* Linn., *Asclepias rosea* R. Br., is a perennial twiner found throughout the plains of the Indian sub-continent near water-logged areas.[1] The plant is used as laxative, laxative, antiseptic, depurative, anthelmintic, antiulcer, aphrodisiac, hepatoprotective and useful in leucoderma and bronchitis. Decoction of plant is used in ulcer, sore-throat and itches. Milky juice is used as galactogogue, antiperiodic, antiulcer and as a vulnerary. Leaves are used as antiperiodic. Its root is prescribed in jaundice. Fruit is bitter, tonic, expectorant, anthelmintic. Fruit juice is used in muscle pain, gonorrhoea, cough and leucoderma, and given to children as astringent.[2,3] The present work deals with not only investigating the laxative activity of the plant, but also finding the most potent extract and performing its phytochemical screening, so as to guide further fractionation of therapeutically potent constituents from this plant.

MATERIALS AND METHODS

Collection and authentication

Oxystelma esculentum in flowering & fruiting stage was collected from Barda Hills near Porbandar, Gujarat, India, in October 2008. Herbarium of the collected sample was prepared and deposited in Department of Pharmacognosy, RK College of Pharmacy (No. RKCP/COG/01/2008). Authentication was done by Dr. N. R. Sheth, Head of Department of Pharmaceutical Sciences, Saurashtra University.

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DOI: 10.5530/pj.2011.24.16

Preparation of extracts

Successive extraction of 1 kg powder of the entire plant was carried out using four solvents in the decreasing order of their polarity index: petroleum ether, chloroform, methanol and distilled water. Complete extraction of the powder with each solvent was carried out in round-bottom flask at a temperature <50°C. The yield of the dried extracts was found to be 10.1%w/w, 8.5%w/w, 7.5%w/w and 14.1%w/w respectively. Their concentrations were adjusted in the solvents according to their dose.

For investigation of each activity, the experimental animals were divided into six groups, with six animals in each group: Normal control, Standard (Agar-agar), Petroleum Ether extract, Chloroform extract, Methanol extract, Aqueous extract.

Pharmacological study

The pharmacological study was approved by the Institutional Animal Ethics Committee (RKCP/COG/RP/10/06) and carried out according to CPCSEA guidelines. All animals were maintained under environmentally controlled conditions of 24 ± 1 °C and 12 h-light and 12 h-dark cycle. The animals were acclimatized to laboratory conditions for 1 month before starting the pre-clinical trials. All studies were performed under standard conditions of temperature, light, humidity and noise.

Wistar rats of either sex weighing 200-220 g were kept in individual cages during one week. Any rat producing wet feces was rejected. The rats were fasted for 12 h before dosing but were given water ad *libitum*. Three animals per group were placed in one metabolic cage (each cage is provided with a wire mesh at the bottom and a funnel to collect the urine; stainless-steel sieves are placed in the funnel to retain feces). Normal control group received normal saline (25 ml/kg). Standard control group received 300 mg/kg Agar-agar (Pharma Pvt. Ltd.) orally. Two groups of three animals were used for each dose of the test extract. Three animals of the test extract groups received orally a dose of 200 mg/kg and the remaining three animals from each of these groups received dose of 400 mg/kg body weight.[4] After administration of the test extracts, the feces were weighed upto 8h and 16h (Table 1, Graph 1).

The same animals were used after a washing period of 4 months for observing the laxative effects of the various extracts in constipated rats. The same procedure was repeated, but Loperamide (Pharma Pvt. Ltd., 5 mg/kg) was used to induce constipation after 1h of administration of each extract. Feces were weighed upto 8h and 16h (Table 2, Graph 2). The results were expressed as the mean (g) of total feces.[5]

Results were calculated as Mean ± Standard Deviation (SD). Statistical analysis of control and test data was performed by One-way ANOVA followed by Dunnett's test (Sigma-stat software). A probability value of $p < 0.01$ was considered statistically significant.

Phytochemical screening

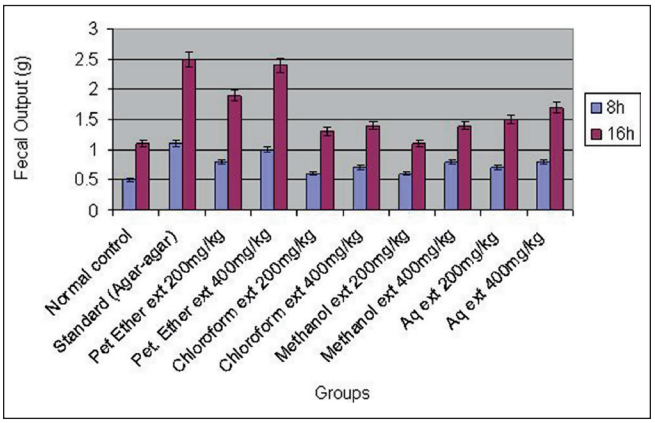
Petroleum ether extract was found to have the most potent and statistically significant laxative activity. This extract was subjected to phytochemical screening involving established methods for detecting various classes of phytoconsituents (Table 3).[6-11]

RESULTS

Table 1: Laxative activity of various extracts in rats

Groups	Fecal Output (g) At 8h	Fecal Output (g) At 16h
Normal control	0.5 ± 0.1	1.1 ± 0.1
Standard (Agar-agar)	1.1 ± 0.1	2.5 ± 0.1
Pet Ether ext 200 mg/kg	0.8 ± 0.1	1.9 ± 0.1
Pet Ether ext 400 mg/kg	1 ± 0.1	2.4 ± 0.1
Chloroform ext 200 mg/kg	0.6 ± 0.1	1.3 ± 0.2
Chloroform ext 400 mg/kg	0.7 ± 0.2	1.4 ± 0.2
Methanol ext 200 mg/kg	0.6 ± 0.2	1.1 ± 0.2
Methanol ext 400 mg/kg	0.8 ± 0.1	1.4 ± 0.2
Aqueous ext 200 mg/kg	0.7 ± 0.1	1.5 ± 0.1
Aqueous ext 400 mg/kg	0.8 ± 0.1	1.7 ± 0.2

Values are expressed as mean ± SD
Number of animals (n) = 6

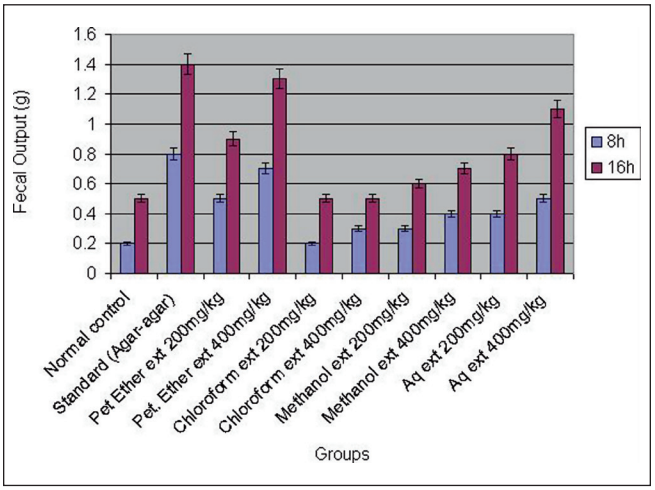


Graph 1: Comparison of laxative potential of various extracts

Table 2: Laxative activity of various extracts in Loperamide-induced constipation model

Groups	Fecal Output (g) At 8h	Fecal Output (g) At 16h
Normal control	0.2 ± 0.05	0.5 ± 0.05
Standard (Agar-agar)	0.8 ± 0.05	1.4 ± 0.05
Pet Ether ext 200 mg/kg	0.5 ± 0.05	0.9 ± 0.05
Pet Ether ext 400 mg/kg	0.7 ± 0.05	1.3 ± 0.05
Chloroform ext 200 mg/kg	0.2 ± 0.1	0.5 ± 0.10
Chloroform ext 400 mg/kg	0.3 ± 0.1	0.5 ± 0.15
Methanol ext 200 mg/kg	0.3 ± 0.08	0.6 ± 0.07
Methanol ext 400 mg/kg	0.4 ± 0.1	0.7 ± 0.15
Aqueous ext 200 mg/kg	0.4 ± 0.06	0.8 ± 0.10
Aqueous ext 400 mg/kg	0.5 ± 0.08	1.1 ± 0.10

Values are expressed as mean ± SD
Number of animals (n) = 6



Graph 2: Comparison of laxative potential of various extracts in Loperamide-induced constipation model

DISCUSSION

The present study shows that the petroleum ether extract of *Oxystelma esculentum* has the most potent, statistically significant and dose-dependent laxative activity amongst all extracts, comparable with agar-agar (Standard) at the dose of

Table 3: Phytochemical screening of petroleum ether extract

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	–ve
	Wagner's test	–ve
	Hager's test	–ve
	Mayer's test	–ve
Flavonoids	Shinoda test	+ve
	Fluorescence test	+ve
Phenolics	Ferric chloride test	+ve
	Folin ciocalteu test	+ve
Sterols and triterpenoids	Libermann Burchardt test	+ve
	Salkowski test	+ve
Carotenoids	Antimony trichloride test	–ve
Cardenolides	Kedde's test	+ve
	Baljet's test	+ve
	Legal's test	+ve

400 mg/kg. Agar-agar exerts its laxative action by accumulation of water in the intestinal loop, increasing the bulk of the stools and stimulating the gastrointestinal motility. Also, loperamide abolishes diarrhea by acting on intestinal motility and consequently reducing the water and stools entering the colon.[12,13] The laxative activity of petroleum ether extract is comparable to agar-agar, indicating a mechanism of action similar to it, thereby overcoming loperamide-induced constipation. This proves the traditional claims of this plant as a potent laxative drug. Phytochemical screening of petroleum ether extract revealed the presence of cardenolides, flavonoids, phenolics, sterols and triterpenoids, which may be responsible for the laxative effect. This bioactivity-guided phytochemical screening can serve as a gauge for further study of therapeutic effects and isolation of therapeutically important compounds from *Oxystelma esculentum*.

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