Cinnamomum camphora (Kapur): Review

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ABSTRACT

Medicinal plants play a vital role for the development of new drugs. The bioactive extract should be standardized on the basis of active compound. The bioactive extract should undergo safety studies. Almost, 70% modern medicines in India are derived from natural products. Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. Camphor is familiar to many people as a principal ingredient in topical home remedies for a wide range of symptoms, and its use is well consolidated among the population of the whole world, having a long tradition of use as antiseptic, antipruritic, rubefacient, abortifacient, aphrodisiac, contraceptive and lactation suppressant. This compound has also a long history of scientific studies on its action and on the way through which it is metabolized in the organisms of both humans and animals, due to the general interest that it has always arisen among common people and scientists.

Key words: Medicinal plants, Camphor, Antiseptic, Abortifacient, Lactation suppressant.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80 % of people in developing countries still relays on traditional medicine, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects which is based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health.[1]

India is sitting on a gold mine of well-recorded and well practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. This can be achieved by judicious product identification based on diseases found in the developed world for which no medicine or only palliative therapy is available; such herbal medicines will find speedy access into those countries. Backward integration from market demands will pay rich dividends. Strategically, India should enter through those plant-based medicines which are already well accepted in Europe, USA and Japan. Simultaneously, it should identify those herbs (medicinal plants) which are time-tested and dispensed all over in India.[2]

Camphor is a natural product derived from the wood of the camphor laurel (*Cinnamomum camphora* L.) trees through steam distillation and purification by sublimination; the

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trees used should be at least 50 years old. Camphor has a counter-irritant, rubefacient and mild analgesic action, and is a major component of liniments for relief of fibrositis, neuralgia and similar conditions. It can be used as a mild expectorant; when ingested camphor has irritant and carminative properties. Camphorated-oil, a solution in oil given through intramuscular or subcutaneous way, can be used as a circulatory and respiratory stimulant, but this use is considered hazardous. When in combination with menthol and chenodeoxycholic acid it has been used to aid dispersal of bile duct stones, although this is no longer recommended. Aim of the present work is to provide an overview over pharmacological, pharmacokinetical and toxicological aspects of camphor, in order to assess its safety profile and evaluate the eventual level of risk connected with its use.[3] Fractionation of the camphor-free oil obtained from *C. camphora* provides an oil rich in safrole (80% or more), usually called Chinese sassafras oil. *C. camphora* is a well-known chemotype; on distillation, the wood from different groups of trees may yield camphor, linalool, safrole or cineole as the major chemical. The use of *C. camphora* as a source of leaf oil has expanded in recent years, and it is now an important source of natural linalool (which is still preferred over the synthetic form for some fragrant applications). The crude oil obtained by primary distillation of the chipped wood is fractionated to remove camphor and provide safrole-rich oil.[4]

Description

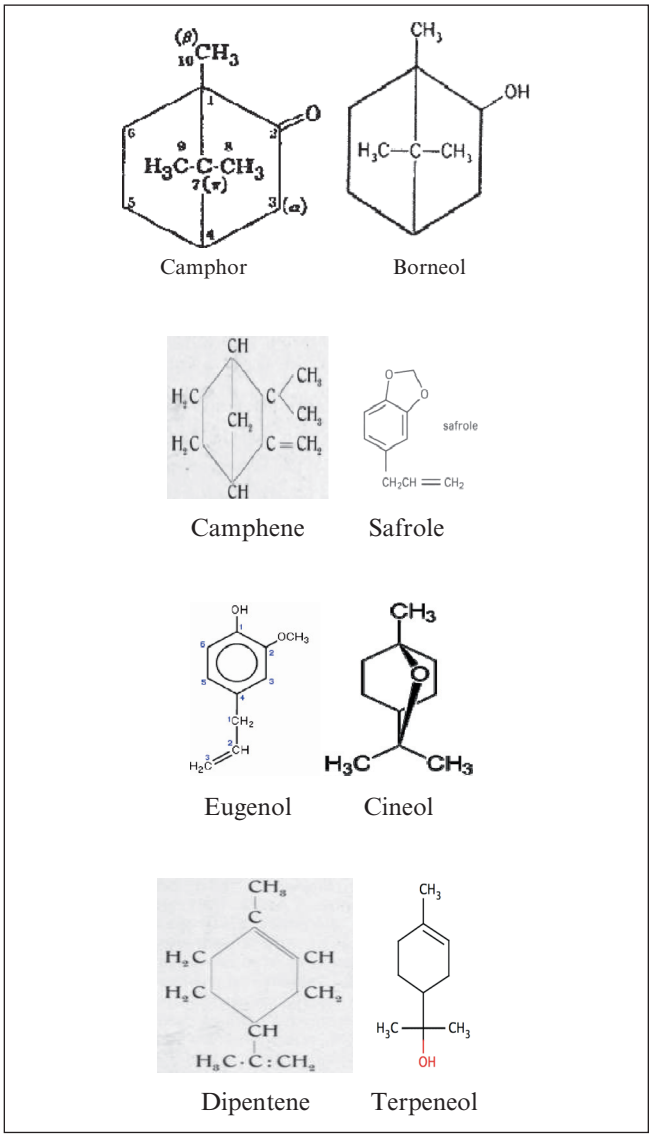
Quite, glabrous. Leaves alternate, chartaceous or finally coriaceous, long petiolate, ovate or oblong, or lanceolate-oblong, at both ends attenuate- acute, 3-nerved, delicately transversely veined on the upper side, shinning, 5-12.5 cm long, 2.5-5 cm broad, uniformly coloured on both sides or below paler or glaucous. Panicles axillary, shorter than the leaf, penducle thin branching at the apex, branches 3-1-flowered, calyx yellow, slightly longer than the pedicel, about 3 mm long, lobes inside slightly cano-tomentose.[5]

Taxonomy of *Cinnamomum Camphora*

Kingdom	–	Plantae
Order	–	Laurales
Family	–	Lauraceae
Genus	–	*Cinnamomum*
Species	–	*C.camphora*
Common name	–	Kapur

Chemical Constituents

The oil's high eugenol content also makes it valuable as a source of this chemical for subsequent conversion into isoeugenol, another flavouring agent. Major oil constituent of *C. camphora* are camphor, linalool, borneol, camphene, dipentene, terpeneol, safrole and cineole.[6]



Medicinal Uses Of *Cinnamomum Camphora*

Camphor has a long history of herbal use. It has been used internally in the treatment of hysteria, but in modern day herbalism it is mainly used as an essential oil and internal use is not advised. The wood and leaves are analgesic, antispasmodic, odontalgic, rubefacient, and are also used as a stimulant. An infusion is used as an inhalant in the treatment of colds and diseases of the lungs. The essential oil, which can be obtained by distillation of the chipped branches, trunk and wood of the tree, or from the leaves and twigs, is the most suitable form of usage. Wood 24 - 40 years old is normally used. The essential oil is anthelmintic, antirheumatic, antispasmodic, cardiotonic, carminative, diaphoretic, sedative and tonic. It is used externally in liniments for treating joint and muscle pains, balms for chilblains, chapped lips, cold sores, skin diseases, etc., and as an inhalant for bronchial congestion. Some caution is advised, excessive use causes vomiting, palpitations,

convulsions and death. It is possible that the oil can be absorbed through the skin, causing systemic poisoning. The essential oil is used in aromatherapy. Its keyword is 'Piercing'. It is used in the treatment of digestive complaints and depression. Sassafras oil from *Cinnamomum camphora* is a source of safrole, which is used to manufacture heliotropin, a valuable flavour and fragrance compound. *C.camphora* is also a source of natural camphor.[6]

Probable Mechanism of Action

Camphor, a natural product derived from the wood of the tree *Cinnamomum camphora*, has a long history of use as antiseptic, analgesic, antipruritic, counterirritant and rubefacient . Its success and wide medical use, especially in topical preparations, is connected to its mild local anesthesizing effect and to the production of a circumscribed sensation of heat, together with its characteristic and penetrating odour that is by most of people associated to the idea of a strong and effective medicine. Camphor is today mostly used in the form of inhalants and of camphorated oil, a preparation of 19% or 20% camphor in a carrier oil, for the home treatment of colds as a major active ingredient of liniments and balms used as topical analgesics. The antitussive, nasal decongestant and expectorant action of camphor and of its derivatives was one of the first ones to be systematically investigated. Its nasal decongesting activity seems to be not purely mechanic, but connected with the stimulation of cold receptors in the nose. The inhalation of camphor vapours (so as the one of eucalyptus and menthol vapours) on a sample of volunteers increased the nasal sensation of airflow through the induction of cold sensation in the nose, despite of actually not affecting nasal resistance to airflow. The analgesic proprieties of camphor are largely known and applied, but little is known about the molecular mechanisms that are at their basis. Demonstrated that camphor activates TRPV3, a member of transient receptor channel superfamily, leading to excitation and desensitization of sensory nerves. The notorious effect of generation of a sensation of heath associated with topic application of camphor is a consequence of this activation. Anyway excessive and repeated application of camphor can lead to sensibilization of TRPV3, in apparent contrast with its analgesic role. The antipruritic, analgesic and counterirritant activity of camphor is instead associated with its capacity of activating TRPV1- another member of TRP channel superfamily - at the level of dorsal root gangliar [DRG] neurons and inhibiting TRPA1 channels , action that is in common with other TRPV1 agonists. Camphor also inhibits other related TRP channels such as ankyrin-repeat TRP1 (TRPA1), which is a further evidence underlying its analgesic effects. Camphor can modulate the activities of hepatic enzymes involved in phase I and phase II drug metabolism. 50, 150 and 300 mg Kg-1 of camphor dissolved in 0.1 ml of olive oil was administered daily to female Swiss Albino mice during 20 days. At its highest concentration it caused a significant increase in the activities of cytochrome P450, cytochrome b5, aryl-hydrocarbon hydroxylase and glutathione S-transferase, significantly elevating the level of reduced glutathione in the liver. Camphor was shown to inhibit mitochondrial respiration. Administration of up to 8 μM of camphor inhibited respiration rate in rat-liver mitochondria, nearly halving the oxygen consumption; this suggests that camphor may be used in oxygenating tumors prior to radiotherapy. Camphor can also be a potential radiosensitizing agent in radiotherapy. D-camphor (1100 μg ml-1) inhibited oxidative metabolism in *E.coli*). Succinic, lactic and NADH-oxidase activities were inhibited, while NADH and succinic DCPIP oxidoreductase enzymes were unaffected. The restoration of succinic oxidase activity by ubiquinone (Q6) but not by vitamin K1 indicates that D-camphor may operate this inhibition by affecting quinone functions.[3]

Interactions

Very few studies of pharmacological interactions between camphor and other compounds are present in literature. In a study combining the administration of D-camphor and an extract from fresh crataegus berries, a synergic action of the two preparations emerged in ameliorating cardiac performances. Both D-camphor and the extract contributed in an increase in total peripheral resistance induced by an increase tone of the arterioles, and while the former appeared to be the main factor in inducing the rapid initial effect, the former added a long-lasting effect.[3]

Known Pharmacological affect of *Cinnamomum Camphora*

1. **Anthelmintic Activity - Investigation of in Vitro Anthelmintic activity of *Cinnamomum Camphor Leaves.*** Infections with helminth are among the most widespread infections in humans and other domestic animals affecting a large number of world population. The majority of these infections due to worms are generally restricted mainly to the tropical regions and the occurance is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symtomps like anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally in population. From the observations, higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Aqueous extract of *Cinnamomum camphora* exhibited anthelmintic activity in dose-dependent manner showing maximum efficacy at 50 mg/ml concentration for all three types of worms. The plant extract exhibited more potent activity at lowest concentration (10 mg/ml) against (roundworm)

Ascaridia galli. Anthelmintic activity of the extract was compared with the standard drug Piperazine citrate.[7]

2. **Antibacterial Activity- Screening Of Antibacterial Sensitivty Of Essential Oils Of Camphor And Cinnamon.** Cinnamon oil was found to be a better antibacterial agent, exhibiting broarange of anti-bacterial activity against common bacteria. Hence, it represents an alternative source of natural antimicrobial substances for use in food systems to prevent the growth of food-borne bacteria extend the shelflife of the processed food. The study also shows that further research on the effects of spices and essential oils on microorganisms can be rewarding to pursue in the search for new broad spectrum antimicrobial agent.[8]
3. **Sperm Motility And Sperm Viability- Effect of *Cinnamomum camphora* on human sperm motility and sperm viability.** Sperm motility is a crucial part of examination for studying the effect of any compound. On microscopic examination, sperms look like a swimming tadpole. Each sperm structure is composed of a head which contains the genetic material of father in its nucleus part, a tail which lashes back and forth to propel sperm along, and a midpiece which has mitochondria (power house of sperm) which provide energy for sperm motion. The presence of sugar fructose produced by seminal vesicles provide energy for sperm motility. this study of effect of camphor on human sperm motility and its viability, a decrease in sperm motility and sperm viability investigated with increased concentration of camphor solution. It may be due decrease in fructose levels, or denaturation of proteins, cholesterol which are indirectly connected with energy source for sperm motility. Thus, it can be indicated that there is a positive relation between Camphor and sperm parameters. Hence, camphor treated samples shows descent in sperm motility count which counts for decreasing effectiveness for fertilization and thus acts as a contraceptive.[9]
4. **Uterus Histology- Effect of Camphor on Uterus Histology of Pregnant Rats.** The study was conducted to investigate the effect of camphor on the rat uterus histology during pregnancy period. In this study, the histological examination for control group uterus (one week gestation) was performed by using the light microscope showed a similar histological structure to rodent uterus at one week gestation. This study was focused on the endometrium (the place of the implantation and placenta formation) where many of the changes could occur during pregnancy. The cellular level showed clear epithelial cells containing oval nucleuses filled by a large amount of chromosomes and is surrounded by granular cytoplasm with regular shape of the outer edge. The results of this research indicate that there is a direct correlation between the amount of the dose used and the negative impact of camphor on the histo-architecture of the uterus of pregnant rats; suggesting negative influence on the reproductive health of the animals which might cause abortion in animals with higher doses of camphor.[10]
5. **Cerebral Cortex Activity- Effect Of Camphor Essential Oil On Rat Cerebral Cortex Activity As Manifested By Fractal Dimension Changes.** Fractal dimension (FD) values of the parietal electrocortical activity were calculated before and after intraperitoneal administration of camphor essential oil (450-675 µl/kg) in anesthetized rats. Camphor oil induced seizure-like activity with single and multiple spiking of high amplitudes in the parietal electrocorticogram and occasional clonic limb convulsions. The FD values of cortical activity after camphor oil administration increased on the average. Only FD values of ictal ECoG sequences were lower than those before camphor oil administration.[11]
6. **Sexual Behaviour- Effects of Camphor on Sexual Behaviors in Male Rats.** According to Iran's folk medicine, camphor, a crystalline ketone obtained from essential oils of *Cinnamomum camphora,* has both sexual behavior attenuating and enhancing properties. The findings of the study showed that there were no significant differences in MF (mount frequency) and IF (intromission frequency) from the control and camphor treated groups. However, there were significant decreases in ML (mount latency) and IL in rats received camphor at a dose of 50 mg/kg compared to that of the control group. The reduction of ML indicates that camphor enhanced sexual desire and motivation, and the reduction of IL is suggestive of enhanced sexual performance. The enhancement of sexual desire by camphor might be mediated through the increase of the synthesis of testosterone in male rats. The peak of plasma testosterone levels in the rat occurs around 50-60 days of age.[12]
7. **Anti-inflammatory and Antioxidative Activity- In vitro anti-inflammatory and anti-oxidative effects of *Cinnamomum camphora* extracts.** *Cinnamomum camphora* Sieb (Lauraceae) has long been prescribed in traditional medicine for the treatment of inflammation-related diseases such as rheumatism, sprains, bronchitis and muscle pains. In this study, therefore, we aimed to investigate the inhibitory effects of *Cinnamomum camphora* on various inflammatory phenomena to explore its potential anti-inflammatory mechanisms under non-cytotoxic (less than 100 µg/ml) conditions. *Cinnamomum camphora* is an active, oriental herbal medicine used in various inflammatory diseases. In spite of its famous legacy, the pharmacological effects have not been fully explored from the immunopathological view point. The modulatory effect of *Cinnamomum camphora* on

macrophage-mediated inflammatory phenomena such as cytokine production, NO release, PGE2 release, functional activation of adhesion molecules and oxidative stress in order to understand its anti-inflammatory action.[13]

8. **Common cold associated with headache- Evaluation of the clinical efficacy and safety of the herbal cold balm in the treatment of the common cold associated with headache.** *Cinnamomum camphora is a plant* which contains a volatile oil comprising of camphor, safrol, linalool, eugenol etc. The oil has antimicrobial activity against common pathogens. It acts as reflex expectorant and is helpful in respiration as well as circulation. Topically it is used as a rubifacient and mild analgesic.[14]
9. **Immunoglobulin E-suppressing Activity- Identification of Dimethylmatairesinol as an Immunoglobulin E-suppressing component of the leaves of *Cinnamomum camphora.*** Immunoglobulin E (IgE) plays an important role in allergic diseases. The study shows that a methanol extract of leaves of the camphor tree *Cinnamomum camphora* reduced the amount of IgE secreted by human myeloma U266 cells. When the methanol extract was fractionated by extraction with organic solvents, the ethyl acetate fraction showed the highest activity. The fraction was further separated into several subfractions by preparative TLC. It was identified that component of one of the active subfractions as dimethylmatairesinol. Thus, the extract of *C.camphora* and its component including dimethylmatairesinol have potential as an anti- allergic agent.[15]

CONCLUSION

Roots, branches, leaves, and wood of *C. camphora* can be used for extracting camphor and camphor oil or pharmaceutical use and as a flavoring. The core of the fruit, which has both industrial and medicinal uses, is approximately 40 percent oil. The wood is used for construction, shipbuilding, and cabinet-making. A large proportion of the world's camphor is now produced synthetically from pinene, a turpentine derivative, or from coal tar. Camphor is used in the manufacture of celluloid, in disinfectants and chemical preparations and has a wide range of medicinal uses. Safrole, produced from the residual oil after camphor extraction, is used in soap and perfume manufacture. *Cinnamomum camphora* has been used medicinally for thousands of years to fight toothache, clear up urinary tract infections and soothe stomach irritation. It has a broad range of historical uses in different cultures including the treatment of diarrhoea, arthritis and various menstrual disorders. The large number of medicinal applications for cinnamon indicates the widespread appreciation of folk herbalists for its healing properties. In the Indian System of Ayurvedic medicine, it is used against a wide spectrum of diseases like bronchitis, colds, congestion, diarrhoea, dysentry, oedema, flu, gas,metabolic and heart strengthening, hiccups, indigestion, liver problems, menorrhagia, melancholy, muscle tension, nausea and vomiting. It assists uterine contractions during labour and menstrual pain from low metabolic function. For external applications, it is used against headaches and pain. In Unani medicine, it is used as a cephalic tonic and cardiac stimulant and for the treatment of coughs. Flowers are used in the European tradition as a blood purifier. *Cinnamomum camphora* may find its way to a diabetic's daily diet. It contains a chemical called methoxy hydroxy chalcone polymer, which can reduce the blood glucose level. *Cinnamomum camphora* is used for religious purposes also.

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Standardization and toxicity evaluation of *Cassia fistula* pod pulp extract for alternative source of herbal laxative drug

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ABSTRACT

The ripe pod pulp of *Cassia fistula* Linn. (Fabaceace) has long been used as a traditional laxative drug due to anthraquinone glycosides constituent. Rhein is the major anthraquinone in the pod pulp. In this study, extracts from ripe pod pulp of *Cassia fistula* collected from 10 different provinces in Thailand were standardized for physical and chemical properties. Using UV-vis spectrophotometric analysis, total anthraquinone content in the pod pulp extracts was more than 0.10% w/w (average 0.1365 ± 0.001% w/w) while total anthraquinone glycosides was more than 0.03% w/w (average 0.0445 ± 0.002% w/w), both calculated in terms of rhein. Quantitative analysis of rhein content in the pod pulp extracts was performed by high performance liquid chromatographic (HPLC) method. It was found that rhein content in pod pulp extracts was more than 0.02% w/w (average 0.0518 ± 0.003% w/w). Acute toxicity of the pod pulp extracts in rats and mice showed no mortality or any signs of toxicity. Total aerobic bacteria count and total fungi count in the extracts did not exceed 10 and 11 cfu/g, respectively while no pathogenic bacteria were found. This information would be a useful guideline for quality control and standardization of *C. fistula* pod pulp extracts for a good quality herbal laxative raw material.

Key words: anthraquinone, *Cassia fistula*, laxative, rhein, standardization

INTRODUCTION

Cassia fistula Linn. (Fabaceae) is a medium-sized, deciduous tree widely grown in tropical and subtropical areas as an ornamental plant for its beautiful, bright yellow flowers (Figure 1). It is native to southern Asia and being the national tree of Thailand. It can be found in every part of Thailand and is locally called "Khun" or "Ratchaphruek".[1] The ripe pods, found during January to April, are mostly thrown away as wastes. *C. fistula* is widely used in folklore medicine for its medicinal properties. The pulp of the ripe pods possesses a mild, pleasant purgative action and is also used as an anti-fungal drug.[2] Some authors have reported antimicrobial, antioxidant, anti-inflammatory, hepatoprotective and hypoglycemic potential of this plant.[3-8] In Ayurvedic medicinal system, *C. fistula* was used against various disorders such as haematemesis, pruritus, diabetes and other ailments.[9,10] In Thai traditional medicines, laxative pill preparations are obtained by boiling the ripe pod pulp of *C. fistula* with water and filtered through muslin cloth, then evaporated to yield the soft extract for making pills.[11]

The ripe pods contain anthraquinone compounds such as rhein, aloe-emodin and sennosides, in both glycosidic and free aglycone forms.[12-15] Anthraquinones are famous for their laxative property of which one of the laxative effects is caused by alteration in colonic absorption and secretion, resulting in fluid accumulation and consequently diarrhea.[16,17] Glycosides of anthraquinones, which are hydrolyzed by β-glucosides enzyme of the intestinal flora to free anthraquinones and further reduced to anthrones, are the active forms responsible for the laxative effect. The potency of the laxative/purgative effect depends on the amount of anthraquinone glycosides.[18,19]

Nualkaew S.[20] previously compared the contents of total anthraquinones and total anthraquinone glycosides in four *Cassia* species, i.e. *C. siamea* Lamk., *C. fistula* Linn., *C. alata* Linn., and *C. surattensis* Burm.f. subsp. surattensis collected from 4 regions of Thailand. The results showed that the leaves of *C. alata* and the pod pulp of *C. fistula* contained the highest amount of total anthraquinone glycosides (1.33%

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Figure 1: Flower and pod of Cassia fistula Linn.

and 0.67%, respectively). Due to the high content of total anthraquinone glycosides and local abundance of *C. fistula* pods in Thailand, this plant can be considered as another natural anthraquinone source for further development as an alternative laxative drug.

Despite the wide range of therapeutic efficacies of *C. fistula*, standardization of the plant extract has not been reported before. Decoction was found to be the appropriate extraction method that promoted the pod pulp extract of *C. fistula* with the highest content of anthraquinone glycosides.[21] Therefore, the purpose of this study was to systematically standardize the ripe pod pulp extracts of *C. fistula*. Chemical properties of the extracts were determined by quantitative analysis of the contents of total anthraquinones and total anthraquinone glycosides (using UV-vis spectrophotometry), and rhein, a major constituent, (using HPLC) in the decoction extracts of the pod pulp of *C. fistula* collected from 10 different provinces in Thailand. TLC fingerprints, loss on drying, solubility, impurities including microbial, heavy metals and pesticides contamination of the pod pulp extracts were also determined. Acute toxicity of the extract was conducted using 2 different animal species to ensure the safety of the extract utilized as a laxative drug. These specifications could be used as a guidance for quality control of the pod pulp extracts of *C. fistula* for pharmaceutical purposes.

MATERIALS AND METHODS

Plant material

The ripe pods of *C. fistula* were collected from 10 provinces in four regions of Thailand: the North (Chaingmai, Nan), the South (Nakhon Si Thammarat), the Central (Ayutthaya, Nakornprathom, Bangkok) and the Northeast (Ubon Ratchathani, Amantcharoen, Mahasarakham, Nakornratchasima) in the summer (April, 2007). They were identified by comparing to those of the herbariums (BKF. No. 118493 and BKF. No. 114900) at The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WCFP001-WCFP010) were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. The ripe pods were cleaned with tap water and the pod pulp (without seed) was separated and kept in a tight container at 4°C until used.

Preparation of pod pulp extracts of *C. fistula* from ten different locations

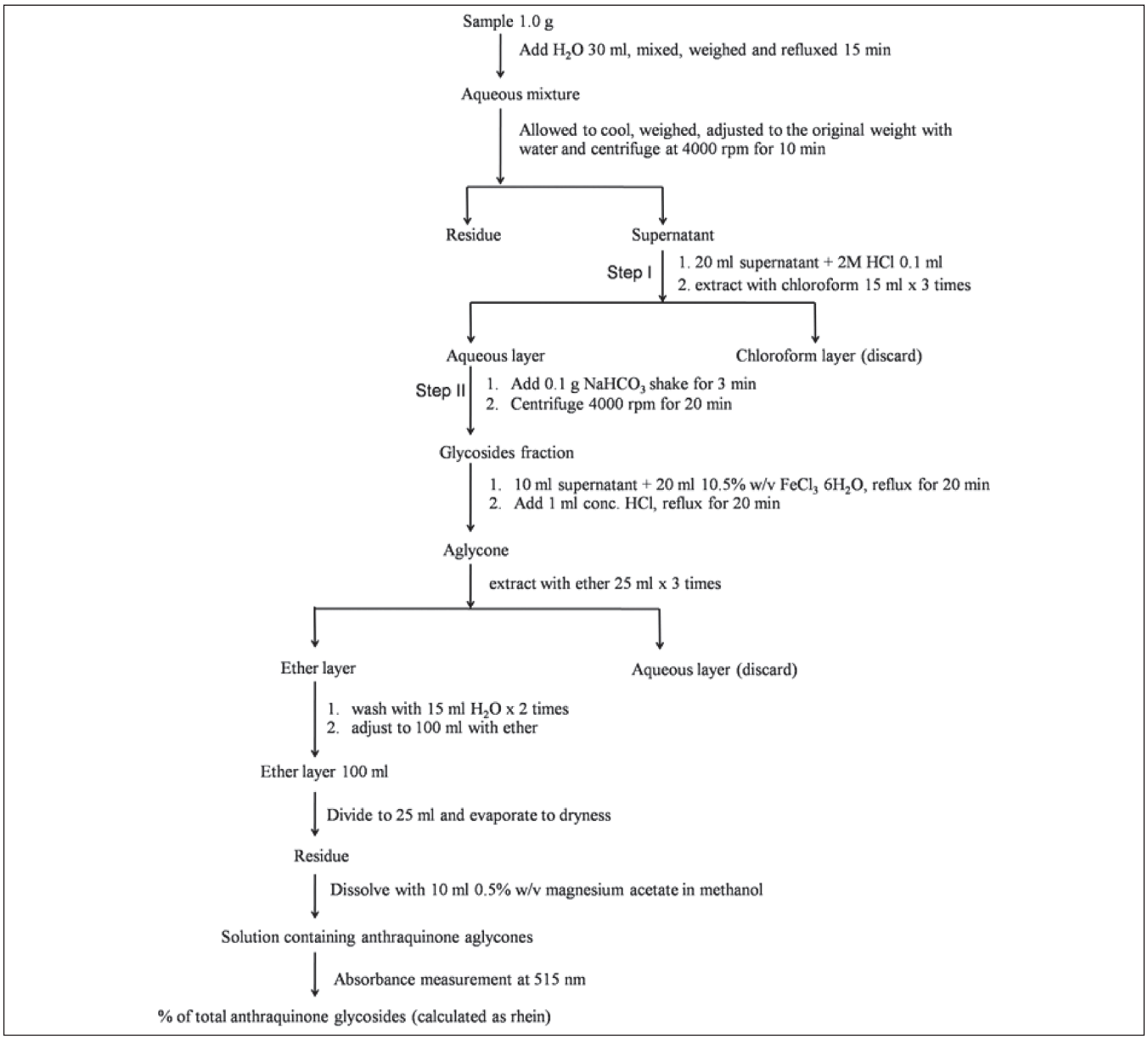
Fresh pulp of *C. fistula* ripe pod (20.0 g) from each location was boiled with distilled water (200 ml) for one hour at 95-98°C and the solution was filtered. The extraction process was repeated until anthraquinones in the pulp were exhaustively extracted (tested by Borntrager's reaction). The filtrates were combined and evaporated to dryness on a boiling water bath to yield a decoction crude extract**.** The yield of crude extract was recorded and the extract ratio (weight of pod pulp:1 g extract) was calculated.

Screening for anthraquinones in *C. fistula* extracts

Anthraquinones in the ripe pod pulp extracts were detected by Borntrager's reaction. Hydrochloric acid (2M) was added to the plant extract, the mixture was heated on a boiling water bath for 15 minutes, then cooled and filtered. The filtrate was liquid-liquid extracted with chloroform. The chloroform layer was separated and shaken with 10% w/v potassium hydroxide solution. The pink-red in upper aqueous layer indicated the presence of anthraquinones.

Quantitative analysis of total anthraquinones and total anthraquinone glycosides in the extracts of *C. fistula* pod pulp from 10 locations using UV-vis spectrophotometric method.

Total anthraquinones and total anthraquinone glycosides contents calculated as rhein in the decoction extracts of *C. fistula* pod pulp from 10 different locations were analyzed using the method modified from the analysis of hydroxyanthracene derivatives of *Cassia alata* described in Standard of ASEAN Herbal Medicine, 1993[22] as shown in Scheme 1. The content of total anthraquinone glycosides was determined by a validated UV-vis spectrophotometry.[21]



Scheme 1: Quantitative analysis of total anthraquinone glycosides in the decoction pod pulp extract.

The analysis of total anthraquinones was performed by the same procedure as total anthraquinone glycosides omitting the extraction of free anthraquinone aglycones (step I and step II in Scheme 1).

Quantitative analysis of rhein content in various decoction extracts from pod pulp of *C. fistula* using HPLC method

HPLC apparatus and conditions

HPLC was performed on a Shimadzu Technologies modular model Class *VP* system consisting of a SCL-10A HPLC system, a UV-vis SPD-10A detector, LC-10 AD and Auto injector SIL-10A (Shimadzu, Japan). The analysis was carried out using a BDS Hypersil C18 column (250 × 4.6 mm, i.d. 5 µm) (Thermo Fisher Scientific Inc., USA) with a BDS Hypersil C18 guard column (10 × 4 mm, i.d. 5 µm) (Thermo Hypersil-Keystone, USA). The isocratic mobile phase was 0.5% aqueous acetic acid solution and methanol (40:60). The total running time was 30 min and a flow rate was 1.0 ml/min. The UV detector monitored at 435 nm while the injection volume was 20 µl.

Calibration curve

Five concentrations of rhein (Sigma, USA) were prepared and diluted with 60% methanol (v/v) in the range of 1.25 - 20.00 µg/ml. Each concentration was performed in triplicate. The calibration curve was obtained by plotting the peak areas from HPLC analysis of each concentration

versus the concentrations of the standard. The regression equation and correlation coefficient (r^2) were then obtained.

Method validation

The HPLC method was validated by evaluation of linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). Rhein was used as a reference standard. Linearity of detector response for standard solutions of rhein was determined by analysis of five concentration levels, each injected in triplicate. Accuracy of the method was confirmed by determination of recovery. The recovery of rhein from the extract was performed on sample spiked with three concentration levels of standard rhein (4, 15 and 20 µg/ml). The precision of the method was assured by analyzing 3 concentrations of standard rhein (n = 3) for intraday precision and on three consecutive days for interday precision. The precision was expressed as percent relative standard deviation (% R.S.D).

Sample preparation

Each decoction extract of *C. fistula* pod pulp (0.2 g) was accurately weighed, dissolved in 60% methanol (v/v) and adjusted to 10 ml in a volumetric flask. All solutions were filtered through a 0.45 µm nylon membrane filter. Each sample solution was analyzed in triplicate.

Physical and chemical properties of *C. fistula* pod pulp extracts

Physical characteristics of the extracts

Color, odor and taste of each extract from the ripe pod pulp of *C. fistula* were determined. All pod pulp extracts had brownish-black with characteristic odor and mild sweet taste.

TLC fingerprints of the decoction extracts of C. fistula pod pulp

TLC fingerprints of the decoction extracts of *C. fistula* pod pulp were performed on a precoated aluminium plate of silica gel $60GF_{254}$ (10 × 20 cm, E-merck, Germany) using ethyl acetate : methanol : water (100:17:13) as the solvent system. The developing distance was 8.0 cm. The plate was dried by an air dryer, and sprayed with 10% alcoholic potassium hydroxide solution. Pink spots on white background of TLC plate indicated the presence of anthraquinones. Rhein, emodin, aloe-emodin, chrysophanol and physcion were used as reference standards.

Loss on drying

Loss on drying of each extract was determined according to the procedure described in Standard of ASEAN Herbal Medicine, 1993.[22] Each sample was done in triplicate and the average weight loss on drying was reported.

Solubility of the extracts

Solubility of the pod pulp extracts in 95% ethanol and in distilled water was investigated at room temperature (25°C). The level of solubility was recorded according to USP 30 NF 25 criteria.[23]

Impurity residues determination

Residues of heavy metals (Cd, Pb, As and Hg) and pesticides (organochlorines, organophosphates, carbamates and pyrethroids) in the extracts were determined according to the AOAC Official Method 999.10 lead, cadmium, zinc, copper and iron in foods[24] and CDFA-MRS method SOP[25], respectively.

Microorganism contamination

Total aerobic bacterial count and total fungi count were determined according to Thai pharmacopoeia supplement, 2005.[26] Some pathogenic bacteria i.e. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Samonella* spp. and *Clostridium* spp. were also examined.

Acute toxicity test

Male and female ICR mice (weight 22-25 g) and both sexes of Wistar rats (weight 130-150 g), from the National Laboratory Animal Centre, Mahidol University, were used in the acute toxicity study. The animals were housed in groups of five per cage for mice and two-three per cage for rats. They were kept in a controlled room (25 ± 1 °C) under 12 hour light/dark cycles for at least one week prior to the experiments. Standard chow and tap water were fed. These experiments were approved by the Institutional Animal Care and Use Committee, Faculty of Pharmacy, Mahidol University (Proof no. PY009/2550).

A Single oral dose (5 g/kg body weight) of the decoction pod pulp extract containing the highest anthraquinone glycoside content (sample collected from Mahasarakham) was administered to both sexes of mice and rats (5/sex/specie) to assess acute toxicity. The control groups (5/sex/species) were administered distilled water. After administration of the extract, all tested animals were examined for the number and time of death within 14 days to estimate the median lethal dose (LD_{50}).

All animals were also observed for sign of gross toxicity and behavioral changes at least once daily during the test period. The observation included gross evaluation of skin and fur, eyes, respiration, circulation, autonomic and central nervous system, motor activity and behavior pattern. Particular attention was directed to salivation, diarrhea, tremor, convulsion and coma. The body weight of all animals was recorded before the experiment and once a week during the test period. Relative toxicity was evaluated according to Lu and Kacew.[27]

RESULTS AND DISCUSSION

Quantitative analysis of total anthraquinones and total anthraquinone glycosides in the extracts of *C. fistula* pod pulp from 10 locations using UV-vis spectrophotometric method

The average extractive ratio of the extracts from ripe pod pulp of *C. fistula* collected from different locations in Thailand (crude drug:1 g crude extract) was 1.7:1 (1.6-1.9:1). Total anthraquinones content in the pod pulp extracts determined by UV-vis spectrophotometry was between 0.1092 ± 0.001 to 0.1646 ± 0.001% w/w (average 0.1365 ± 0.001% w/w) calculated as rhein while the amount of total anthraquinone glycosides was in the range of 0.0330 ± 0.002 - 0.0624 ± 0.001% w/w (average 0.0445 ± 0.002% w/w) calculated as rhein (Table 1).

Quantitative analysis of rhein content in the decoction extracts from pod pulps of *C. fistula* using HPLC method

The proposed HPLC method for analyzing the content of rhein in the pod pulp extracts of *C. fistula* showed acceptable validation parameters. The correlation coefficient (r^2) value of standard rhein was ≥ 0.999, confirming the linearity of the method. The average R.S.D. value lower than 2% and the average recovery of rhein of 99.51% indicated the precision and accuracy of the method, respectively. The LOD and LOQ for rhein were 0.112 and 0.340 µg/ml indicating a high sensitivity of the method. Determined by the validated HPLC condition, the content of rhein in the pod pulp extracts was in the range of 0.0291 ± 0.002 - 0.0726 ± 0.003% w/w (average 0.0518 ± 0.003% w/w) calculated as rhein (Table 1).

Table 1: Yield, extract ratio, contents of total anthraquinones and total anthraquinone glycosides in *C. fistula* pod pulp extracts calculated as rhein and content of rhein.

				Content of total anthraquinoes and total anthraquinone glycosides calculated as rhein using UV-vis spectrophotometric method (% w/w)[a]		Content of rhein by HPLC (% w/w)[a]
Part	province	Yield (% w/w)[a]	Extract ratio	Total anthraquinones	Total anthraquinone glycosides	Content of rhein
North	Nan	56.88 ± 0.16	1.8:1	0.1570 ± 0.002	**0.0330 ± 0.002**	0.0298 ± 0.002
	Chaingmai	61.61 ± 0.07	1.6:1	0.1325 ± 0.001	0.0480 ± 0.002	0.0293 ± 0.001
North-East	Ubonratchathani	56.28 ± 0.29	1.8:1	0.1273 ± 0.001	0.0353 ± 0.001	0.0547 ± 0.002
	Amnatcharoen	62.59 ± 0.43	1.6:1	0.1146 ± 0.001	0.0439 ± 0.003	0.0604 ± 0.003
	Mahasarakham	56.43 ± 0.58	1.8:1	0.1617 ± 0.001	0.0624 ± 0.001	0.0681 ± 0.003
	Nakhonratchasima	58.94 ± 0.10	1.7:1	0.1342 ± 0.001	0.0448 ± 0.001	0.0460 ± 0.002
Central	Ayutthaya	58.50 ± 0.04	1.7:1	0.1353 ± 0.003	0.0360 ± 0.001	0.0663 ± 0.004
	Nakornprathom	60.28 ± 1.33	1.7:1	0.1283 ± 0.001	0.0466 ± 0.003	0.0291 ± 0.002
	Bangkok	71.31 ± 3.02	1.4:1	0.1092 ± 0.001	0.0356 ± 0.002	0.0617 ± 0.003
South	Nakhon Si Thammarat	52.49 ± 0.04	1.9:1	0.1646 ± 0.001	0.0598 ± 0.001	0.0726 ± 0.003
Average		59.53 ± 0.61	1.7:1 (1.6-1.9:1)	0.1365 ± 0.001	0.0445 ± 0.002	0.0518 ± 0.003

[a]expressed as mean ± SD (n = 3)

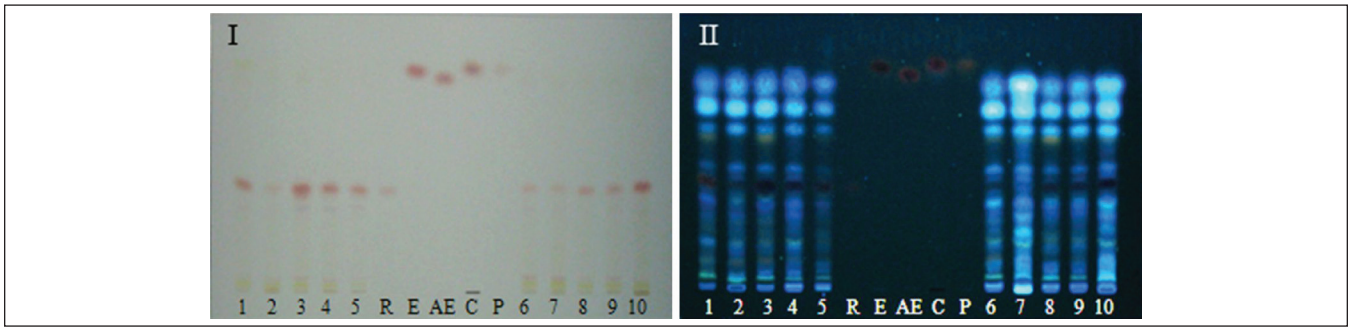


Figure 2: TLC fingerprints of decoction extracts of *C. fistula* pod pulp from 10 different locations. I sprayed with 10% KOH, II detected under UV 365 nm

1 = Ubon Ratchathani, 2 = Amnatcharoen, 3 = Mahasarakham, 4 = Chaingmai, 5 = Nakornratchasima, 6 = Nan, 7 = Nakhon Si Thammarat, 8 = Ayutthaya, 9 = Nakornprathom, 10 = Bangkok, R = Rhein, E = Emodin, AE = Aloe-emodin, C = Chrysophanol, P = Physcion.

From the results, the average contents of anthraquinones in the decoction extracts from the pod pulps of *C. fistula* from 10 locations inferred that total anthraquinones and total anthraquinone glycosides should be more than 0.10% and 0.03% w/w, respectively while rhein content should be more than 0.02% w/w. All pod pulp extracts had brownish-black color with characteristic odor and mild sweet taste. The extracts gave the positive results, signifying the presence of anthraquinones by Borntrager's test and thin layer chromatography. TLC fingerprints of all pod pulp extracts showed similar chromatographic patterns with the major bands corresponded to rhein with the hRf value of 36 (Figure 2).

Loss on drying of pod pulp extracts was less than 1% w/w (average 0.44 ± 0.11 % w/w). The extracts were freely soluble in water but slightly soluble in 95% ethanol. Heavy metals and pesticide residues, and microbial contamination are shown in Table 2. Cadmium and arsenic were not detected in the decoction extracts but lead and mercury were detected at less than 0.27 and 0.01 ppm, respectively. Total bacteria count in the extracts was not more than 10 cfu/g while total fungi count was not more than 11 cfu/g. Pathogenic bacteria such as *S. aureus, E. coli, P. aeruginosa, Salmonella* spp. and *Clostridium* spp. were not found in the extracts. The pesticide residues such as organochlorines, organophosphates, pyrethroids and carbamates were not detected in the extracts (detection limits for organochlorines, organophosphates, pyrethroids and carbamates were 0.01, 0.01-0.04, 0.05 and 0.01 mg/kg, respectively). Amounts of heavy metal residues, microbial contamination and pesticide residues were below the limits recommended by Thai Pharmacopoeia, 2005[26] and Thai Herbal Pharmacopoeia, 2000.[28]

Acute toxicity test showed that the decoction extract of 5 g/kg body weight did not cause any mortality or any sign of toxicity to the treated animals after administration and during the observation period of 14 days. The body weight gain of all animals was not significantly different between the treated groups and the respective control group (Table 3). LD_{50} of the extract after a single oral administration was more than 5 g/kg body weight for all tested animals. Therefore, the extract could be classified as slightly toxic extract.[27] This result supported the former report of Akanmu et al.[29] that *C. fistula* pod infusion was safe for consuming as a laxative drug.

Akiremi et al.[30] carried out biological assays using white male albino rats to compare the laxative activity of *Cassia acutifolia* (Senekot® tablet) and *C. fistula* pod. From the results,

Table 2 : Characteristics, loss on drying, heavy metal and pesticide residues, and microbial contamination of *C. fistula* pod pulp extracts

Test	Results	Limit allowed for dried herbal raw materials[a]
Color	Brownish-black	
Odor	Characteristics	
Solubility in water	freely soluble	
Solubility in 95% Ethanol	slightly soluble	
Average loss on drying (% w/w)	0.44 ± 0.11	
Heavy metal residues (ppm)		
Cd	ND[b]	< 0.3
Pb	< 0.27	< 10
As	ND	< 4
Hg	< 0.01	<0.5
Pesticide residues (ppm)	ND	0.01-1
Organochlorine group	ND	
Organophosphate group	ND	
Pyrethroid	ND	
Carbamate groups	ND	
Microbial contamination (cfu/g)		
Total aerobic bacteria count	< 10	5.0×10^7
Yeast and mold count	< 11	5.0×10^4
E. coli	non	< 5.0
S. aureus	non	non/1 g
P. auruginosa	non	non
Salmonella spp.	non	non/10 g
Clotridium spp.	non	non/10 g

[a]According to Thai Herbal Pharmacopoeia, 2000 [28] and Thai Pharmacopoeia Supplement, 2005 [26]
[b]ND = not detected

Table 3: Comparison of the average weight of the control group and that of the tested group in both genders of rats and (mice) during the test period

Groups	gender	Body weight of rats and mice (grams)[a]		
		0 week	1st week	2nd week
Control group	male	143.00 ± 2.02 (29.00 ± 0.55)	191.50 ± 2.70 (32.00 ± 0.63)	219.60 ± 3.12 (33.40 ± 0.87)
	female	128.00 ± 1.10 (24.40 ± 0.24)	157.40 ± 2.27 (26.20 ± 0.37)	169.80 ± 3.02 (27.80 ± 0.58)
Tested group	male	141.00 ± 1.82 (28.20 ± 0.66)	187.20 ± 2.67 (29.80 ± 0.73)	203.80 ± 6.08 (32.20 ± 0.85)
	female	126.80 ± 1.10 (24.80 ± 0.37)	157.20 ± 2.92 (26.40 ± 0.51)	164.80 ± 1.50 (28.00 ± 0.55)

(...) Body weight of mice, [a] expressed as mean ± SD

C. fistula pod infusion appeared to be as potent as *C. acutifolia* infusion (reference drug). The mean percentages of wet feces produced in 12 hours after being administered infusion of *C. fistula* pod and *C. acutifolia* at the dose 500 mg/kg are 57.3 and 50.7%, respectively. Thus, *C. fistula* pod pulp extract could be used as an alternative laxative drug as same as Senna.

Being the first report on the physical and chemical specifications of *C. fistula* pod pulp decoction extract, this information would be useful as a guideline for quality control and standardization of *C. fistula* pod pulp extracts to be used as a herbal laxative raw material. According to the traditional use for a laxative drug [11], the recommended dose is 4-6 g per day of fresh pod pulp, which was equal to 2.4-3.6 g of the decoction extract. This dose contains about 1.068-1.602 mg of anthraquinone glycosides (equivalent to 1.2432-1.8648 mg of rhein). The pod pulp of *C. fistula* has long been used as a safe traditional laxative drug and the safety was confirmed by our toxicity test results. Furthermore, the pods of *C. fistula* are abundantly found as wastes in all parts of Thailand and many other countries. Like senna extract, it would be economically and pharmaceutically beneficial to promote the standardized *C. fistula* pod pulp extract for herbal laxative products.

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Pharmacognostic Analysis of Stem Bark of *Combretum albidum* G. Don; An Unexplored Medicinal Plant

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ABSTRACT

Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. Certain tribal people of Chinnar, Idukki District of Kerala are using stem bark of *Combretum albidum* G.Don as an effective remedy for jaundice. The documentation of traditional knowledge from Chinnar area reveal that it is highly effective. So the validation and standardisation of stem bark of *C. albidum* was carried out to establish its macro- and microscopical standards, physicochemical parameters, preliminary phytochemical investigation and TLC profiles to evaluate the characters of the plant. The stem bark is usually hard with longitudinal cracks, around 0.5 to 1 cm in thickness, internally bright yellow in colour, odourless and slightly bitter in taste. Detailed anatomy showed the presence of cork, cortex and secondary phloem embedded with elongated lignified phloem fibres. Histochemical studies revealed the presence of lignin, tannin, oil and starch grains. Physicochemical parameters like moisture content, total ash, alcohol and water-soluble extractive values were also evaluated. Preliminary phytochemical analysis indicated a high percentage of tannins, flavonoids and triterpenes and this may be one of the reasons behind the hepatoprotective activity of this plant. Well resolved TLC profiles were recorded in sequential extraction with petroleum ether, ethyl acetate and methanol. The above parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material used for Jaundice by Chinnar tribals of Kerala.

Key words: *Combretum albidum*, histochemical study, pharmacognostic standardisation, physico-chemical parameters.

INTRODUCTION

As a result of the adverse effects associated with synthetic drugs, people started looking back at the ancient healing systems like Ayurveda, Siddha and Unani. Herbal drugs play an important role in health care programs especially in developing countries. However, obstacle behind the acceptance of alternative medicines in developed countries is the lack of documentation and stringent quality control. So the documentation and standardization of the raw materials used in herbal medicine is very essential for the worldwide acceptance of this system of medicine. Correct identification and quality assurance of plant material is indispensable to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy. Pharmacognostic standardisation of plant material include its morphological (organoleptic), anatomical and biochemical characteristics[1].

The Muthuvans, Chinnar tribe of Idukki district, Kerala is using many plants for their health care and day to day ailments but many of them are not yet scientifically validated. This tribe is using a plant called Manjakody as an effective remedy for jaundice. It is identified as *Combretum albidum* G.Don of the family combretaceae. Though *Phyllanthus amarus* and *P. airy-shawii* are very common there and they are aware of the use of these plants, they prefer water extract of stem bark of *C. albidum* as a remedy for both normal and severe jaundice[2]. As far as the available literature is concerned, this plant has not yet been scientifically validated. *C. albidum*, Buffalo calf plant in English, is a large woody climbing, deciduous shrub, up to 30 m high. Its distribution is restricted to semi-evergreen and deciduous forests, along river banks of Peninsular India and Sri Lanka. In the present study, pharmacognostic standardisation of stem bark of *Combretum albidum* was carried out to establish its macro- and microscopical standards, physicochemical parameters, preliminary phytochemical investigation and TLC profiles to characterise the plant material.

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MATERIALS AND METHODS

Collection and Authentication

The plant collected from Chinnar, Idukki district of Kerala was authenticated at Taxonomy Division, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala. The voucher specimen (Col. No. 5545) and raw drug (Col. No. CMPR/RD 211) were deposited at CMPR herbarium and raw drug depository respectively. One part of heart wood was preserved in Formalin: Acetic acid: Alcohol mixture (FAA) for anatomical studies and the remaining part shade dried and powdered for the estimation of physico-chemical parameters and preliminary phytochemical investigation.

Pharmacognostic Standardization

Organoleptic characters such as shape, size, colour, odour, taste and fracture of stem bark were determined. Microscopic studies were carried out by preparing thin hand section of stem bark cleared with chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine[3,4]. Histochemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material[5].

Physico-chemical Evaluations

Moisture content, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as described in Indian Pharmacopoeia[6].

Preliminary Phytochemical Screening

The methanolic extract of stem bark was subjected to tests for the presence or absence of the major class of compounds by standard methods[7].

TLC profile

Powdered stem bark, 10 g was extracted by refluxing with petroleum ether, ethyl acetate and methanol (50 ml × 3) sequentially for a period of 30 minutes each and the combined extract of each were filtered and concentrated to 10 ml. Apply 10 µl each extract as bands at a height of 10 mm from the base of a 5 × 10 cm precoated silica gel Aluminium plate 60 F_{254} using CAMAG Automatic sampler (ATS4) and developed up to 80 mm from the base of the plate in an Automatic Developing Chamber (CAMAG ADC2) using the mobile phase hexane: ethyl acetate (9:1) v/v for petroleum ether, chloroform: ethyl acetate: formic acid (8:2:1) v/v for ethyl acetate and ethyl acetate: formic acid (9:1) v/v for methanol extract. Dry the plate in air and profile pictures were taken in CAMAG documentation visualizer attached with DXA252 camera under UV 254 nm, 366 nm and in visible light after derivatization with anisaldehyde sulphuric acid reagent (ANS).

RESULTS AND DISCUSSION

Morphological characters: Drug occurs in pieces of variable size and thickness, surface rough due to longitudinal cracks and raised lenticels, recurved, channelled to half quilled, greyish to dark brown externally and internal surface fibrous and bright yellowish in colour (Figure 1), odourless, slightly bitter in taste; on drying, the stem bark becomes curved and the rhytidoma gets separated from the bark.

Anatomical characters: Mature bark showed 8 to 15 layers of cork consisting of tangentially elongated storied cells and dead tissues of rhytidoma. The cells of the outer and inner cork are lignified, varying in size and shape filled with yellow brown contents. The cortical cells are thin-walled embedded with stone cells and prismatic crystals of calcium

Figure 1: ***Combretum albidum*** **G.Don**

oxalate. Phloem fibre thick-walled, highly lignified and arranged in radial bands of broad centre and pointed tips with prismatic crystals of calcium oxalate; crystal fibres containing prismatic crystals of calcium oxalate in each chamber. Medullary rays multiseriate, wavy, dialated towards outer side, composed of thin-walled, radially elongated cells (Figure 2).

Powder microscopy: powder showed surface view of cork cells; groups of stone cells; fibres with reticulate thickening; stone cells; tannin masses; group of pitted parenchyma; fragments of fibres and groups of crystal fibres; cortical parenchyma containing tannin and oil globules; yellowish brown content, starch grains and prismatic crystals of calcium oxalate are scattered throughout the powder (Figure 3).

Physicochemical parameters: The value of loss on drying at 110^0 showed the presence of moisture content in the sample, which is 10.29%. The total ash and acid insoluble ash were found to be 7.17% $\pm$ 0.09 and 0.16% $\pm$ 0.08 respectively. The ash contents showed the amount of inorganic matter present in the sample and the acid insoluble ash almost within 1%, which expresses low siliceous matter present in the sample. Extractive values of ethanol and

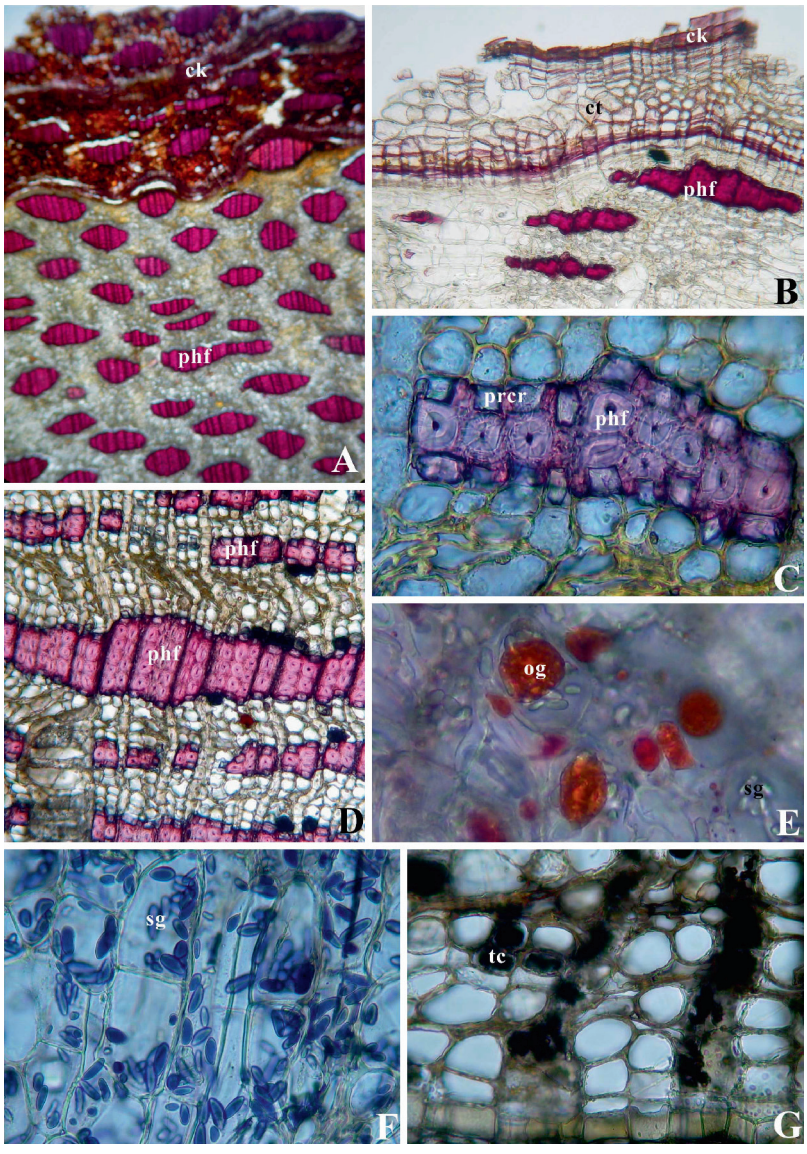

Figure 2: Microscopy of *C. albidum* stem bark. **A** & **B.** TS of bark showing cork and phloem region × 40 & × 100. **C & D.** Histochemical test for lignin × 400 & × 100. **E**. Histochemical test for oil globules; **F**. Histochemical test for starch × 400. **G**. Histochemical test for tannin deposits × 400.
Abbreviations
ck, cork; og, oil globules; phf, phloem fibres; prcr, prismatic crystals of calcium oxalate; sg, starch grains; tc, tannin contents.

water were 6.25% and 10.09% respectively. Sequential extractive values of sample in petroleum ether, ethyl acetate and methanol were 0.43%, 0.65% and 6.82% respectively (Table 1).

Preliminary phytochemical investigation: Methanol extract of stem bark showed presence of tannins, flavonoids, triterpenes, saponins and glycosides. Steroids and alkaloids were found to be absent (Table 2). Flavonoids, triterpenoids and tannins are well known for their hepatoprotective activities[8-10]. The plant possesses a high percentage of tannins, flavonoids and triterpenes and this may be one of the reasons behind the hepatoprotective activity of this plant.

TLC profile: Well resolved TLC profiles were recorded for the future reference and identity of the plant material.

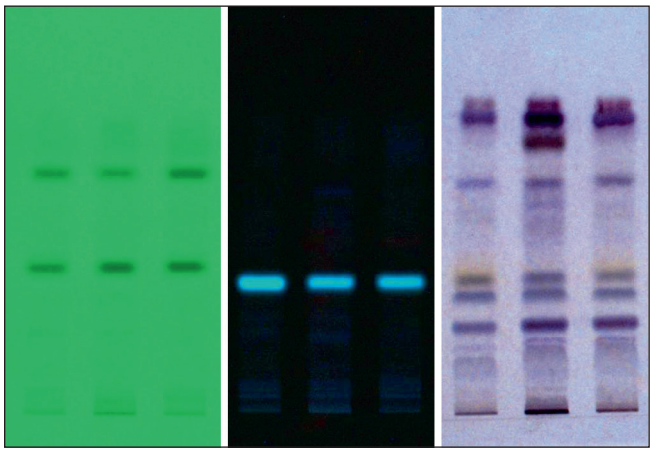

Figure 3: Powder microscopy of *C. albidum* stem bark. **A** & **B**, surface view of cork cells; **C**, groups of stone cells; **D**, prismatic crystals of calcium oxalate; **E**, fibres with reticulate thickening; **F** & **H**, yellowish brown content; **G** & **J**, stone cells; **K** & **I**, tannin masses; **L**, group of pitted parenchyma; **M**, fragment of fibre; **N**, cortical parenchyma containing tannin; **O** & **P**, groups of crystal fibres; **Q**, starch grains; **R**, cortical parenchyma containing oil globules.

Petroleum ether extract TLC profile showed two spots under UV 254 nm, one spot under UV 366 nm and five spots after derivatization in visible light (Figure 4 & Table 3). In ethyl acetate extract the profile showed six spots under UV 254 nm, four spots under UV 366 nm and nine spots after derivatization (Figure 5 & Table 4). In methanol extract, TLC profile showed six spots under UV 254 nm, two spots under UV 366 nm and six spots after derivatization with ANS (Figure 6 & Table 5).

All the above parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material used for Jaundice by Tribals of Kerala.

CONCLUSION

WHO has emphasised the need to ensure quality control of the raw materials used for herbal medicines by using

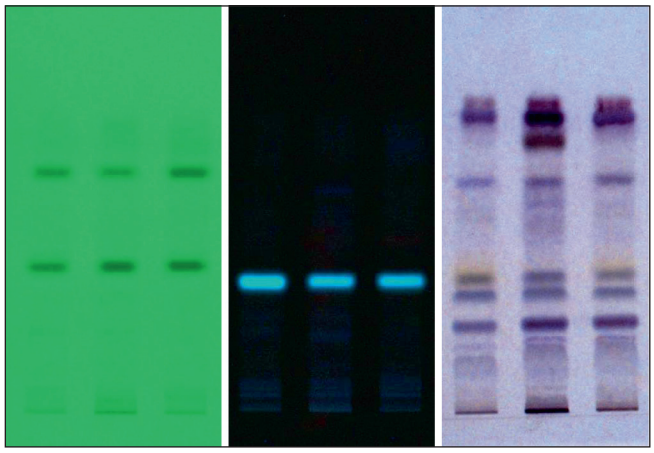

Figure 4: TLC details of *Petroleum ether* extracts of *C. albidum* stem bark. A. UV 254 nm; B. UV 366 nm; C. Derivatized with ANS.

Table 1: Physicochemical analysis of stem bark of *C. albidum*

Physicochemical constants (%)	**Total ash**	**Acid-insoluble ash**	**Moisture content**
	7.17 ± 0.09	0.16 ± 0.08	10.29 ± 0.13
Extractive values (%)	**Ethanol**	**Water soluble**	
	6.25	10.09	
Sequential extractive values (%)	**Petroleum ether**	**Ethyl acetate**	**Methanol**
	0.43	0.65	6.82

Table 2: Preliminary phytochemical investigations *C. albidum* stem bark

Test for active constituents	Tannins	Flavonoids	Triterpenes	Glycosides	Saponins	Steroids	Alkaloids
	+++	++	+	+	+	–	–

–Absent; + presents

Table 3: TLC details of petroleum ether extract of stem bark of *C. albidum*

Detection/Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R_f values	Colour of the band	R_f values	Colour of the band	R_f values	Colour of the band
Track 1, 2 & 3	0.45	Grey	0.40	Blue	0.27	Violet
	0.75	Grey			0.36	Greyish blue
					0.42	Greyish blue
					0.45	Yellow
					0.72	Violet

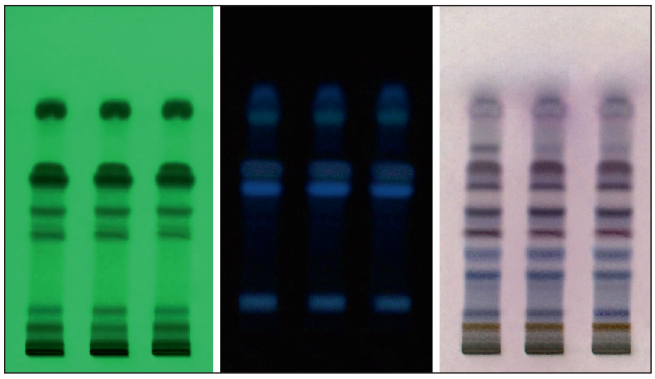

Figure 5: TLC details of *Ethyl acetate* extracts of *C. albidum* stem bark. A. UV 254 nm; B. UV 366 nm; C. Derivatized with ANS.

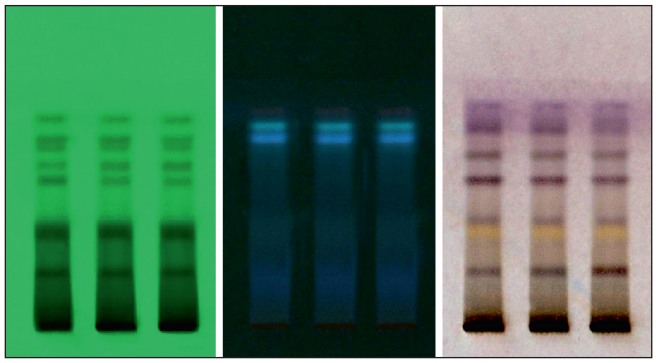

Figure 6: TLC details of *Methanol* extracts of *C. albidum* stem bark. A. UV 254 nm; B. UV 366 nm; C. Derivatized with ANS.

Table 4: TLC details of ethyl acetate extract of stem bark of *C. albidum*

Detection/Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R_f values	Colour of the band	R_f values	Colour of the band	R_f values	Colour of the band
Track 1, 2 & 3	0.10	Grey	0.19	Blue	0.10	Brown
	0.16	Bluish grey	0.61	Blue	0.16	Bluish grey
	0.43	Grey	0.67	Greyish blue	0.30	Bluish grey
	0.52	Grey	0.85	Greenish blue	0.36	Bluish grey
	0.64	Grey			0.45	Ash
	0.69	Grey			0.52	Ash
					0.62	Ash
					0.68	Greyish brown
					0.74	Ash

Table 5: TLC details of methanol extract of stem bark of *C. albidum*

Detection/Visualization	Under UV 254 nm		Under UV 366 nm		After derivatization	
	R_f values	Colour of the band	R_f values	Colour of the band	R_f values	Colour of the band
Track 1, 2 & 3	0.23	Blackish grey	0.77	Blue	0.24	Dark brown
	0.40	Blackish grey	0.82	Greenish blue	0.40	Yellow
	0.60	Grey			0.44	Pale grey
	0.65	Grey			0.60	Dark brown
	0.73	Grey			0.71	Pale grey
	0.76	Grey			0.80	Violet

modern techniques and by applying suitable parameters and standards. In the present study various standardisation parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation and TLC profiles in petroleum ether, ethyl acetate and methanol extracts were studied, which are being reported for the first time in this plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of stem bark of *Combretum albidum* G. Don for the future reference.

ACKNOWLEDGEMENT

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Extraction process optimization of tylophorine from *Tylophora asthmatica* Wight & Arn.

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ABSTRACT

Tylophora asthmatica (Synonym- *Tylophora indica*; family Asclepiadaceae) is a popular traditional remedy for respiratory disorders. The plant contains phenanthroindolizidine type of alkaloids like tylophorine and tylophorinine. The present study was designed to optimize extraction process of tylophorine from *T. asthmatica* leaves. A series of extraction experiments were carried out using different solvents like 95% alcohol, 2% sulphuric acid solution, chloroform, defatting with petroleum ether then extraction with 95% alcohol, 95% alcohol with different concentrations of glacial acetic acid (1%, 2%, 5% & 10%) and citric acid (0.1%, 0.5%, 1%, 2% & 5%). Maceration method was used for extraction. From all these experiments the extracts obtained were concentrated, purified by acid-base purification technique and tylophorine content was determined. The pure tylophorine fraction was analyzed by HPLC method. Results of the above study show that extraction with 95% alcohol containing 2% citric acid was most effective in terms of higher yield of tylophorine and economy. Hence this method may be used commercially for extraction of tylophorine.

Key words: *Tylophora asthmatica*, Tylophorine, optimization process.

INTRODUCTION

Tylophora asthmatica (Synonym-*Tylophora indica*) is a climbing perennial plant indigenous to India and known as Antamul in Hindi. It grows wild in the southern and eastern regions. It belongs to family Asclepiadaceae and leaves are mainly used for medicinal purposes[1]. The leaves are traditionally used as an expectorant, anti-dysenteric and to treat bronchial asthma, allergic rhinitis, bronchitis, rheumatism, and dermatitis. In the later half of the 19th century, it was called *Indian ipecacuahna,* as the roots of the plant have often been employed as an effective substitute for ipecac. Its use as an emetic led to *Tylophora's* inclusion in the Bengal Pharmacopoeia of 1884[2].

Apart from its traditional uses, it has recently been found to possess strong anti-inflammatory activity, anti-allergic and antispasmodic actions[3]. *Tylophora* has become an increasingly popular treatment for asthma, based on its traditional use for this purpose, and several pharmacological studies performed in the 1970s. It contains 0.2-0.3% of alkaloids having phenanthroindolizidine moiety. Tylophorine and Tylophorinine are the major alkaloids present and the percentage is not affected by seasonal variations[4-6]. Tylophora extracts have shown antiinflammatory[7] and anti cancer avtivities[8]. Tylophora extracts and active constituent-tylophorine have demonstrated antiasthamatic activity in experimental animals and in clinical studies[9-12].

Tylophorine is the main bioactive component from *T. asthmatica*[13-14]. The plant is used commercially however, the extraction process is not economically feasible. Hence the objective of the present study was to establish an optimized extraction process from *T. asthmatica* leaves to get maximum yield of tylophorine.

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MATERIALS AND METHODS

Plant material

Tylophora asthmatica leaves were procured from Ecotech Technologies (I) Pvt. Ltd., Mumbai. The leaves sample was identified from Botanical Survey of India, Northern circle, Dehradun. Reference No. – BSI/NC-9(Tech)/06-07/487.

Chemicals and reagents

All solvents, reagents & chemicals used were of AR and HPLC grade (Rankem, SD Fine chemicals, E Merck or Qualigens fine chemicals India). Distilled water and

Table 1: List of different extracting solvents used in various extraction processes

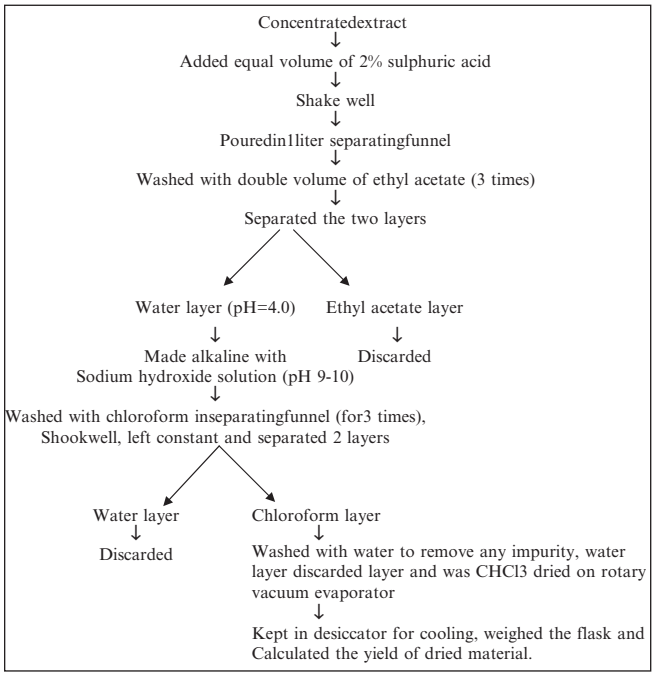
Experiment no.	Extraction solvent
1	Maceration with 95% alcohol containing 10% glacial acetic acid
2	Defatting with petroleum ether, then maceration with 95% alcohol
3	Maceration with 2% Sulphuric acid
4	Maceration with 95% alcohol
5	Basified with lime then maceration with chloroform
6	Hot extraction with 95% alcohol
7	Hot extraction with 95% alcohol containing 2% glacial acetic acid
8	Hot extraction with 95% alcohol containing 5% glacial acetic acid
9	Hot extraction with 95% alcohol containing 2% citric acid
10	Hot extraction with 95% alcohol containing 5% citric acid
11	Extraction at room temperature with 95% alcohol containing 2% glacial acetic acid
12	Hot extraction with 95% alcohol containing 0.5% citric acid
13	Hot extraction with 95% alcohol containing 0.1% citric acid
14	Hot extraction with 95% alcohol containing 1.0% citric acid

precoated silica gel G plates (20 × 20 cm, 0.2 mm thickness, aluminium base, E Merck) were used.

Extraction process

To optimize extraction method a total of fourteen extraction experiments were performed using different solvents. Maceration extraction method was used on laboratory scale. In each experiment 100 g of *T. asthmatica* leaves powder was taken and extraction was performed five times. For each extraction 500 ml of solvent was employed. A list of extraction experiments is given in table 1.

The extract obtained from each experiment was concentrated to one fourth of its total volume on rotary vacuum evaporator and then purified by further acid-base purification technique:



In the extraction experiment where citric acid was used for extraction, the concentrated extract was not acidified with 2% sulphuric acid solution, because melting point of citric acid is above 100 °C and boiling point of ethanol is near 70 °C hence citric acid does not evaporate during the concentration of extract. So the concentrated extract is already acidic.

Pilot batch extraction

The extraction process with optimum extraction of tylophorine was selected and a pilot batch extraction was performed with 6 kg *T. asthmatica* leaves with the selected method.

Standardization of isolated fraction

The isolated fraction was identified by thin layer chromatography technique as tylophorine alkaloid when compared with standard tylophorine. It was characterized by various spectroscopic techniques like UV, IR, NMR and Mass and analyzed by HPLC technique to determine its tylophorine content. Reverse phase analytical HPLC was employed with following conditions:

Column: Hypersil BDS, C18 (4.6 × 250) mm, 5 um
Flow rate: 1.0 ml/min.
Injection volume: 10 µl
Detection: UV-260 nm
Mobile phase:

Solvent A- 20ml Glacial acetic acid + 2ml Tri ethyl amine in 1000ml water
Solvent B- Acetonitrile

Mobile phase (Gradient) composition for *Tylophora* leaves extract

Time (minute)	Solution A (%)	Solution B (%)
00.00	90	10
30	60	40
35	40	60
40	30	70
45	40	60
50	90	10
55	90	10

Sample preparation: 10 mg/ml in glacial acetic acid

Results

T. asthmatica mature leaves were selected for present study because it was determined first that mature leaves contains maximum amount of tylophorine alkaloid as shown in table 2.

The results of various extraction experiments performed for optimization of extraction method for *T. asthmatica* leaves are given in table 3.

From the above results it was concluded that hot extraction with 95% alcohol containing 2% citric acid gave best yield of tylophorine. This method was selected for pilot batch extraction of 6 kg *T. asthmatica* leaves. The following results were obtained with pilot batch extraction:

Extraction equipment used= Stainless steel circulatory extractor
Weight of final concentrated extract obtained = 5.418 Kg
Volume of concentrated extract obtained = 5.0 liter (approx)
Weight of compound obtained after drying of chloroform layer = 14.463g
Weight of compound obtained after absolute alcohol treatment = 3.843g (0.064%w/w)
Batch no. assigned to the compound = TAT-E-07/023
Tylophorine content in TAT-E-07/023 = 75.8% w/w (by HPLC analysis)

Table 2: Tylophorine content in fresh plant parts of *Tylophora asthmatica*

S. No.	Plant part	Tylophorine content (%w/w)
1	Young leaves	0.066
2	Mature leaves	0.074
3	Old leaves	0.012
4	Stem	0.008

TLC finger print profile of isolated tylophorine

Test preparation - 5mg of each extracted dried compound was dissolved in 2.5ml of chloroform.
Standard preparation - 1mg of reference material was dissolved in 1.0ml of chloroform.
Spotting - 10µl of test sample was applied as band on precoated TLC plates using micro syringe.

Development of plate

Mobile phase: Toluene: Ethyl acetate: Diethylamine (7:2:1).[15]
Detection: UV 254 nm without spray reagent

(S = Tylophorine standard, TAT-E-07/023= Tylophorine extracted by selected method)

TLC finger print profile of Tylophorine extracted using optimized method

CHARACTERIZATION OF TAT-E-07/023
Yield = 0.064%w/w
HPLC chromatographic purity = 75.8%w/w
IR spectra of TAT-E-07/023: (These all spectral data are found comparable with available spectral data for Tylophorine structure.)

Table 3: Results of various extraction experiments

Experiment no.	Total alkaloid Yield (g)	Tylophorine content (%w/w) (By HPLC analysis)	Total Tylophorine yield (g)
1	0.217	20.18	0.0403
2	0.085	19.61	0.0166
3	0.159	16.05	0.0255
4	0.115	19.35	0.0222
5	0.076	34.84	0.0264
6	0.131	19.84	0.0259
7	0.130	22.80	0.0296
8	0.101	21.39	0.0216
9	0.280	17.25	0.0483
10	0.197	17.17	0.0338
11	0.176	11.86	0.0208
12	0.129	19.17	0.0247
13	0.121	20.57	0.0248
14	0.169	21.89	0.0369

IR (KBr, Vmax cm^{-1}):
3437.57, 2930.73, 2830.31, 1618.40, 1513.81, 1426.80, 1246.45, 1196.83, 1149.60, 1018.26, 843.10.

1H-NMR spectra of TAT-E-07/023:

1H- NMR ($CDCL_3$, 400MHz,δ ppm): δ
1.70-2.25 (4H, br, m, H-12 & H-13)
2.90 (1H, t, J = 26.1 Hz, H-11)
3.36 (1H, dd, J = 2.57 Hz & 18.25 Hz, H-13a)
3.46 (1H, t, J = 15.29 Hz, H-11)
3.64 (1H, d, J = 13.81 Hz, H-9)
4.05 (6H, S, 2-OMe & 7-OMe)
4.11 (6H, S, 3-OMe & 6-OMe)
4.60 (1H, d, J = 14.68, H-9)
7.14 (1H, S, H-1)
7.29 (1H, S, H-8)
7.81 (2H, S, H-4 & H-5)

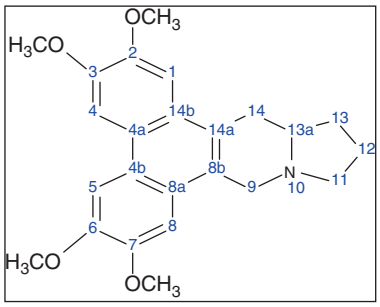
13C-NMR spectra of TAT-E-07/023:

13C-NMR ($CDCL_3$, 400MHz, δppm):
103.701(C-1), 149.253(C-2 & 3), 104.010(C-4), 104.546(C-5), 148.966(C-6), 149.039(C-7), 103.895(C-8), 54.5520(C-9), 34.3050(C-11), 22.1685(C-12), 31.8139(C-13), 55.6919(C-14), 126.390(C-4a), 126.514(C-4b), 123.977(C-8a), 124.167(C-8b), 60.7605(C-13a), 124.892(C-14a), 126.840(C-14b), 56.4183(C-2MeO & 3MeO) and 56.5885(C-6MeO & 7MeO).

Mass spectra of TAT-E-07/023:

MS (M+): 393 (M^+), 324 (Base peak)

Based on the above spectral data and comparison with standard the structure of extracted alkaloid was confirmed to be tylophorine.



Structure of Tylophorine

Standardization of *Tylophora asthmatica* leaves extract by HPLC:

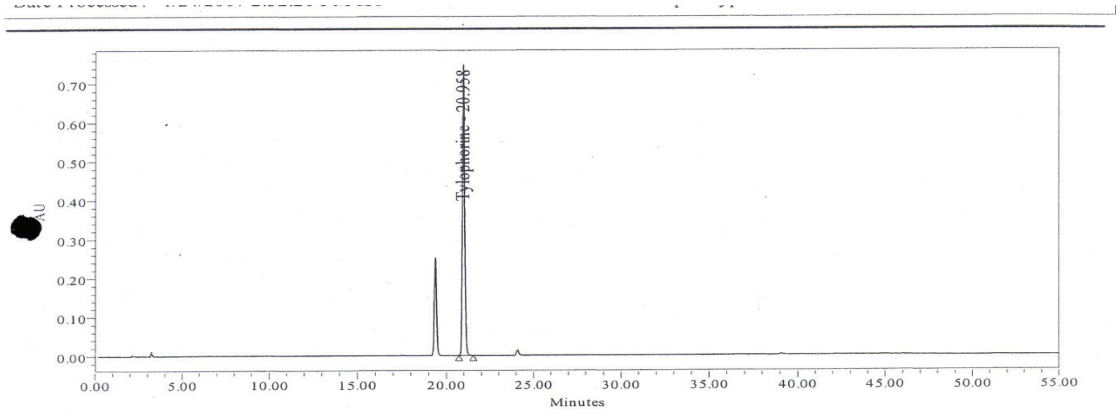
Estimation of tylophorine

Test compound = TAT-E-07/023

HPLC chromatographic purity = 75.8%w/w

CONCLUSION

Alkaloids are the main constituents of *Tylophora asthmatica* leaves. The main alkaloid is tylophorine which is responsible for the anti-asthmatic effect of the plant. In these extraction experiments, highest yield of tylophorine was obtained with 95% alcohol containing 2% citric acid because alkaloids reacts with acid and form salt and the salt form is more soluble in 95% alcohol and higher yield is obtained. Results of the above study show that extraction with 95% alcohol containing 2% citric acid was most effective in terms of higher yield of tylophorine and economy. Hence this method may be used commercially for extraction of tylophorine



	SampleName	RT	Area	SampleWeight	Dilution	Amount (%)	Int Type
1	Test_B.No.TAT-P-02/013	20.96	7009492	10.84	10000.00	69.3	BB

HPLC chromatogram

HPLC chromatogram of TAT-E-07/023

	Sample name	RT	Area	Sample weight	Dilution	Amount (%)	Int type
1	Test B.No.TAT-E-07/023	20.96	7009492	10.84	10000.00	75.8	BB

from *T. asthamatica*. HPLC method has not been reported so far for determining the tylophorine content; hence this may be used for determining the purity of isolated tylophorine.

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Pharmacognostical Studies of Seeds on Some Plants Belonging Chhattisgarh

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ABSTRACT

Diospyros melonoxylon Roxb. belonging to family Ebenaceae, commonly known as Tendu in Hindi is a small tree with rather slender stem and smooth grey bark. *Buchanania lanzan* spreng. commonly known as Char in Hindi is a tree of 12-15 mt high, with straight trunk, belonging to family Anacardiaceae. *Manilkara zapota* (Linn.) P. Royen syn., belonging to family sapotaceae commonly known as Chiku in Hindi is a large, evergreen, forest tree more than 30 mt in height. These three plants are easily available in the forests of Chhattisgarh used in various diseases traditionaly. The pharmacognostical studies of these plants were done by evaluating its microscopical studies, phytochemical screening, flourescence analysis of extracts of the seeds with different reagents and presence of Elemental analysis such as CHNS and heavy metals. The microscopical studies revealed the specific characters in all three seeds, it has obsereved and identified. Phytochemical screening revealed the presence of carbohydrates, proteins, fat and oils, present in extracts of all three seeds. Flourescence analysis of Extarcts of seeds shows diffrent colours with different reagents. The above pharmacognostical and preliminary phytochemical studies will be beneficial for proper identification and authentification of seeds of *D. melonoxylon* Roxb., *B. lanzan* spreng, *M. zapota* (Linn.) P. Royen syn.

Key words: Pharmacognostic study, *Diospyros melonoxylon*, *Buchanania lanzan*, *Manilkara zapota*

INTRODUCTION

Diospyros melonoxylon Roxb [Image 1a]. commonly known as Tendu in Hindi, a small tree with rather slender stem and smooth grey bark belonging to family Ebenaceae.[1] The air dried seeds contain reddish yellow oil, 6.0; unsaponificable matter, 2.1; iodine value, 8.5; and saponificable value, 200. It is a rich source of unusual cyclopropenoid fatty acids, 24 % and shows sufficient promise for its exploitation for industrial utilization. The fatty acid composition of the oil is as follows: palmitic, 13.7; stearic, 5.5; oleic, 18.5; linoleic, 11.9; keto acid, 29.0; malvalic, 12.7; and sterculic acid, 8.7 %.[2] *D. melanoxylon* leaves, extensive chemical screening resulted in the isolation of nine pentacyclic triterpenes and β-sitosterol.[3,4] The extracts of *D. melanoxylon* showed significant antihyperglycemic activity as compared to standard drug.[7] The fruit is bitter, acrid; cooling, digestible, carminative, astringent to the bowels; The leaves have bad taste and smell; diuretic, carminative, laxative, styptic; good in epistaxis and night blindness; improve the eyesight; used in opthalmina, trichiasis, burns, tuberculosis gland, scabies, and old wounds. The dried flowers are aphrodisiac, diuretic; useful in leucorrhoess, urinary discharges, inflammation of the spleen, scabies, night blindness, and anemia; enrich the blood. The bark of the tree possesses astringent properties, and is used as decoction in diarrhoea and dyspepsia as tonic,[5] antimicrobial activity,[6] antihyperglycemic activity,[7] anti-plasmodial activities against chloroquine-sensitive (3D7) and chloroquine-resistant (K1) strains of P. falciparum.[8]

Buchanania lanzan spreng [Image 1b] commonly known as Char (Hindi), Charoli (Gujarati) is a tree 12-15 mt high, with straight trunk, belonging to family from Anacardiaceae.[1] The kernels contains moisture, 3.0; protien, 19.0; fat,59.0; fibers, 3.8; carbohydrates 12.1; and minerels 3.0 g/100 gm; calcium, 279.0; phosphours, 528.0 (phytin phosphorus, 158.0); iron, 8.5; oxalic acid, 2.0; magnisum, 373.0; sodium, 10.2; potasium, 436.0; copper, 0.86; sulphur, 186.0; chorine,

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25.0; thiamine, 0.69; riboflavin, 0.53; niacin, 1.5; vitamins C, 5.0 mg/100; lipid 65.6 % comprised mainly of neutral lipids 90.4 % consisting mostly of triacylglycerols 82.2 %, free fatty acid 7.8 % and small amount of diacylglycerols, monoacylglycerols and sterols.[2] The leaves contain quercetin, gallic acid, kaempferol, kaemferol-7-O-glucoside, quercetin-7-O-rhamnoside, quercetine-3-O-rhamnogluvosside and myricetin 3-rhamnoside. Two major class of secondary metabolites were detected Glycosides, Phenolic compounds from leaves.[9] Glycoside, myricetin 3-rhamnoside-3-galactoside, has been isolated from leaves of *B. lanzan*. Its structure was established from spectroscopic and chemical evidence.[10] The root is acrid; removes biliousness; cures blood diseases. The fruit is sour, sweet, fattening, laxative, binding cooling, aphrodisiac; cures biliousness, fevers, thirst, ulcers, blood diseases. The oil is sweet; indigestible. The juice of the leaves is digestive, expectorant, aphrodisiac, purgative; purifies the blood; allayss thirst; lessens biliousness. The seed have a slightly bitter pleasent taste; expectorant, tonic to the body and the brain, stomachic; remove bad humors; useful in gleet and urinary concretions; good in fevers; cause headache. [5] The oil extracted from the kernels of the fruit is used as a substitute for almond oil in native medicinal preparation and confectionery. It is also used as anti-inflammatory and antioxidant,[11] antiulcer.[12]

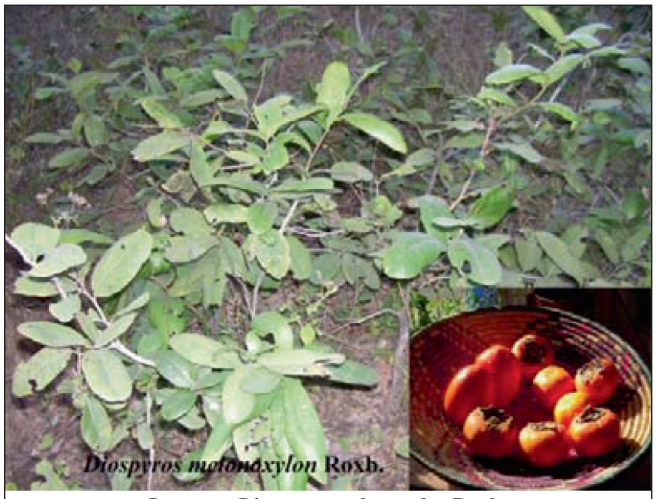

Image a: ***Diospyros melonoxylon*** **Roxb.**

Image b: ***Buchanania lanzan*** **spreng.**

Image c: ***Manilkara zapota*** **(Linn.) P. Royen syn.**

Image 1: Various Plant of Chhattisgarh

Manilkara zapota (Linn.) [Image 1c] commonly known as Chickle tree (English), Chiku (Hindi) is a large, evergreen, forest tree more than 30 mt in height a tree belonging to family from Sapotaceae.[1] The leaves contain crude protien, 19.03; hemicellulose 20.04; cellulose 11.50; neutral detergent fibre 46.06; acid detergent fibre 26.02; and silica 0.25 of dry matter. The fruit conttain fructose, α and β glucose and sucrose. The seeds contain protien (8 %) amino acid composition as leucine 8.0; lysine 4.1; methionine 0.4; phenylalanine 3.0; and valine 5.8 %. Plant is rich sources of Saponin, tannin, sugars, proteins, ascorbic acid, phenolics, carotenoids and minerals like iron, copper, zinc, calcium, potassium and vitamins like folate, niacin and pantothenic acid.[2] The seeds are aperients, diuretic, tonic and febrifuge. The bark is antibiotic, astringent and febrifuge. The fruit are bulk laxative, anti-diarrheal, haemostatic, anti-haemorrhoidal, antiemetic, anti-rheumatic and in kidney and bladder stones.[5] The plant is also useful as antimicrobial,[13] antibacterial activity against 10 Gram positive, 12 Gram negative bacteria and one fungal strain, Candida tropicalis.[14]

MATERIALS AND METHODS

Material

Plant collection and authentication

The fruit were selected for all the plant and seed was separated from the fruit. The plant was authenticated by Prof. H. B. Singh, NISCAIR/RHMD/consult/2010-11/1664/215,262, Dated 22/12/2010, 14/02/2011, New Delhi (India). The

voucher No. of specimen HNSIPER/Herb/10, HNSIPER/Herb/11 and HNSIPER/Herb/12 for *M. zapota* (Linn.) P. Royen syn. *B. lanzan spreng* and *D. melonoxylon* Roxb. respectively was deposited at Pharmacognosy department of Shree HNSIPER, Rajkot, Gujarat, India.

Method

Extraction

The seeds were dried and coarsely powdered and extracted first with water then with either acetone/methanol by heating under reflux. The extracts were concentrated under reduced pressure to a semisolid mass and it was made free from solvent. Extracts of all seeds were subjected for fluorescence analysis and detection of heavy metals.[15]

Microscopy analysis

Microscopic studies were carried out by preparing of thin hand section of leaf (camera 130UMDModel). The sections were cleared with alcohol and stained as per the protocol. Histochemical reaction were applied with concentrated Hydrochloric acid and pholoroglucinol were mounted in glycerine for identification of lignified elements, iodine solution for identification of starch grains, Sudan red-III for identification of Cutin.[15,16]

Phytochemical investigation

The acetone and methanolic extracts of all the seeds were subjected to further preliminary, qualitative phytochemical investigation.[16]

Fluorescence analysis

Fluorescence analyses of extracts of all seeds were carried out by standard methods. It was observed under day light and UV light (254nm and 365nm).[17,18]

Determination of ash value

The total ash, acid-Insoluble ash, water soluble ash and sulphated ash were determined as per the guideline of Ayurvedic Pharmacopoeia of India.[19]

Mineral Elemental Analysis Present in Ash

Ash of plant consists of Inorganic element which can be easily detected by chemical tests. The element like Ca^{++}, Mg^{++}, Na^{+}, Ka^{+}, Fe^{++}, SO_4^{-}, Cl^{-}, CO_3^{-} I, P, and N remain unchanged in ash.[16]

Elemental composition (CHNS) and Heavy Metal Analysis

Elemental composition (CHNS) were analyzed by using elemental analyzer (Elemental, Vario E.L, Hanau Germany, at C and M Lab, central Ashwamedh Engineers and consultants Co-operative Society (Ltd), Nasik, Maharashtra)[20-24] and heavy metals on extracts of all seeds was carried out by standard method.

RESULTS AND DISCUSSION

The results of extractive value are tabulated in Table 1. Results indicate the percentage yield is higher for *B. lanzan* then *M. zapota* and finally the least for *D. melonoxylon*

Microscopical Characteristics

The transverse section of the seed of *D. melonoxylon* is irregularly circular shaped. The testa consists of single layered irregular rectangular sclerenchyamatous cells. Testa is followed by irregularly arranged parenchyamatous cells containing brownish matter. Endosperm is composed of simple irregular parencyamatous cells surrounding the embryo [Figure 1].

The transverse section of the seed of *B. lanzan* is irregularly circular shaped. The testa consists of several layered irregular sclerenchyamatous cells. Testa is followed by endosperm consist of simple rounded parencyamatous cells surrounding the embryo [Figure 2].

The transverse section of the seed of *M. zapota* is oval shaped. The testa consists of several layered irregular sclerenchyamatous cells. Testa is followed by endosperm consist of irregular parencyamatous cells surrounding the embryo [Figure 3 and 4].

Table 1: Technological Characterization of Percentage Yield

Extract	Percentage Yield
EDM	2.0
EBL	16.5
EMZ	5.9

EDM- Extract of *Diospyros melonoxylon*, EBL- Extract of *Buchanania lanzan*
EMZ- Extract of *Manilkara zapota*

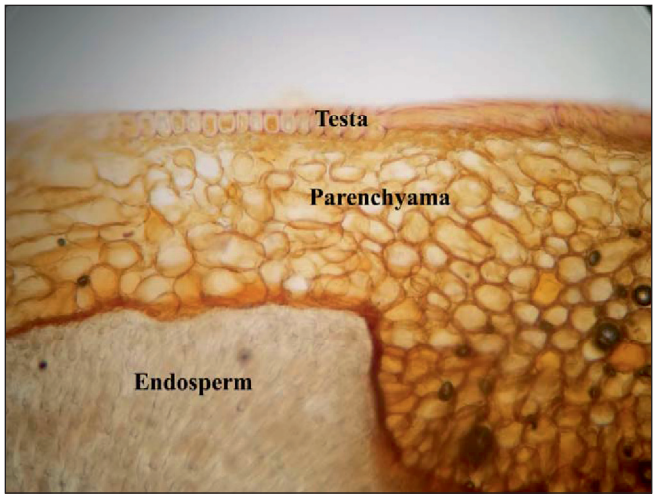

Figure 1: Transverse section of seed of *Diospyros melonoxylon*

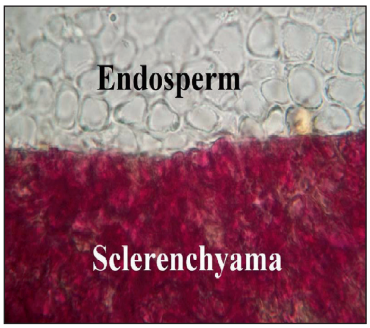

Figure 2: Transverse section of seed of Buchanania lanzan

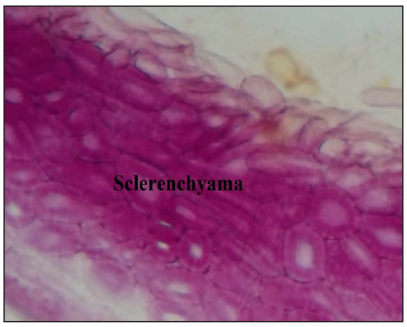

Figure 3: Transverse section of seed coat of *Manilkara zapota*

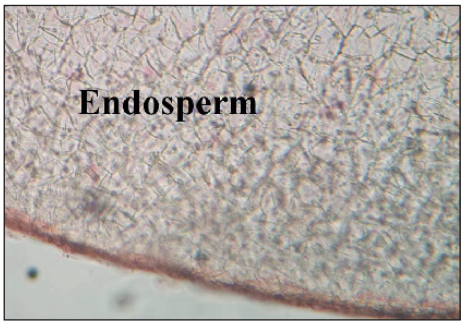

Figure 4: Transverse section of seed (endosperm) of *Manilkara zapota*

Table 2: Phytochemical Investigation of Seeds Extracts

Plant constituent	Extracts					
	D. melonoxylon		*B. lanzan*		*M. zapota*	
Test/Regent Used	**AE**	**ME**	**AE**	**ME**	**AE**	**ME**
Alkaloids						
Mayer's Regent	–	–	–	–	–	–
Dragendorff//'s Regent	–	–	–	–	–	–
Hager's Regent	–	–	–	–	–	–
Wagner's Regent	–	–	–	–	–	–
Carbohydrates						
Molish's Test	+	+	+	+	+	+
Fehling solution	+	+	+	+	+	+
Benedict's Regent	+	+	+	+	+	+
Barfoed's Regent	–	–	–	–	–	–
HCl solution	–	–	–	–	–	–
Selwinoff's Regent	–	–	–	–	–	–
Tollen's Phlorogucinol	+	+	+	+	+	+
Iodine Solution	–	–	–	–	–	–
Tannic acid Solution	–	–	–	–	–	–
Protein						
Biuret Regent	+	+	+	+	+	+
Million's Regent	+	+	+	+	+	+
Amino Acid						
Ninhydrin Regent	–	–	–	–	+	+
Million's Regent	–	–	–	–	+	+
Test for cysteine	–	–	–	–	–	–
Fats and Oils						
$KHSO_4$ Solution	+	+	+	+	+	+
$CuSO_4$ Solution	+	+	+	+	–	+
Phytosterols						
Salkowski Regent	+	+	–	–	–	+
Libermann Burchard Reagent	+	+	–	–	–	+
Libermann's Regent	–	–	–	–	–	–
Glycosides						
Keller-Killiani Regeant	–	+	–	+	–	+
Shinoda Test	–	+	–	+	–	+
Phenolic Compound & Tannins						
Ferric chloride solution	+	+	–	–	+	+
Acetic acid solution	+	+	–	–	+	+
Dilute HNO_3 solution	–	–	–	–	–	–
Dilute NH_4OH solution	–	–	–	–	–	–
Saponins	–	–	–	–	–	–
Mucilage's						
Ruthenium Red	+	+	+	+	+	+
Aqua's KOH	+	+	+	+	+	+

* AE: Acetone Extract, ME: Methanolic Extract, + Present, – Absent.

Table 3: Fluorescence Analysis of EDM, EMZ & EBL Seeds

Treatment	Day light			UV (254nm)			UV (365nm)		
	EDM	EMZ	EBL	EDM	EMZ	EBL	EDM	EMZ	EBL
Powder as such	Brown	Light brown	Light Yellow	Dark Brown	Dark Brown	Dark Yellow	Greenish	Greenish	Yellow
Powder + Dil.HNO_3	Brown	Light brown	Reddish brown	Yellow	No color	Green	Black	Light green	Dark Brown
Powder + Dil. HCl (1N)	Dark reddish brown	No color	Dark Green	Yellow	No color	Brown	Black	No color	Dark Brown
Powder + Conc.H_2SO_4	Reddish brown	Blackish brown	Dark Brown	Dark green	No color	Yellow	Black	Light green	White
Powder + Ammonia	Maroon	No color	Light yellow	Fluorescence green	No color	Greenish brown	Black	Black	Greenish White
Powder + Iodine solution	Reddish	Reddish brown	Bluish White	Parrot color	Parrot color	Light Blue	Black	No color	Dark Blue
Powder + $BaCl_2$ (10%)	Buff color	Light grey	Grey	No color	Light green	No Color	No color	Light green	No Color
Powder+ NaOH (Aq.40%)	Dark brown	Light brown	Light Green	Light green	Light green	Green	Light green	Green	Pinkish Brown
Powder+$AgNO_3$ (10%)	Grey	No Fluorescence	No Fluorescence	No Fluorescence	No color	No Color	No Fluorescence	No color	No Color
Powder + $FeCl_3$ (5%)	Bluish black	Brownish black	Brown	No Fluorescence	Light green	Dull Green	No Fluorescence	Green	Black
Powder+$MgSO_4$ (10%)	Light brown	No color	No Color	No Fluorescence	No color	No Color	No Fluorescence	Light green	No Color
Powder+ $FeSO_4$ (10%)	Light brown	Light brown	Brown	Green	No color	No Color	Green	Light green	No Color
Powder + $CaCl_2$ (5%)	Light brown	No color	No Color	No color	No color	No Color	No color	No color	No Color
Powder + Lead acetate (40%)	Grey	No color	Light Grey	No color	No color	No Color	No color	No color	No Color
Powder + $CuSO_4$ (5%)	Green	Light green	White Green	No color	No color	No Color	No color	Black	Dark Black
Powder + Bromine water	Light brown	No color	Brown	Light green	Light green	Green	Light green	Black	Dark Green
Powder + Potassium Ferrocyanide (2%)	Brown	Dark brown	Brown	Light green	Parrot color	Light Green	Light green	No color	Light Blue
Powder +Ammonium thiocyanate (5%)	Light brown	Light brown	Brownish White	Light yellow	No color	No Color	Light yellow	Light green	Green
Powder +Ammonium molybdate (10%)	Dark brown	No color	No color	Light yellow	No color	No Color	Light yellow	Black	Light Black
Powder + Sodium Cobalt nitrite (30%)	Maroon	maroon	Light Red	No color	Light green	Green	No color	No color	No color

EDM- Extract of *Diospyros melonoxylon*, EBL- Extract of *Buchanania lanzan* EMZ- Extract of *Manilkara zapota*

Phytochemical Investigation

The phytochemical tests of acetone and methanolic extracts of all the seeds revealed presence of various phyto-constituents [Table 2].

Table 4: Technological Characterization of Ash Analysis of Seed Powder.

Ash Analysis	EDM	EBL	EMZ
Total ash content (%)	16.1	15.4	17.25
Acid insoluble ash (%)	02.25	01.3	01.5
Water soluble ash (%)	0.625	0.54	0.96
Sulphated ash (%)	11.25	10.9	11.31

EDM- Extract of *Diospyros melonoxylon*, EBL- Extract of *Buchanania lanzan*
EMZ- Extract of *Manilkara zapota*

Fluorescence Analysis

The results of fluorescence analysis tabulated in [Table 3]. The extracts of all the seeds produce different colours and fluorescence in under day light and UV light (254nm and 365nm) with various reagents.

Ash Analysis

Ash values used to determine quality and purity of crude drug. Ash simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a from adulteration. Ash value indicative to some extent of care taken in collection and preparation of drug from market and of foreign matter content of natural drug. Results indicate that the various ash contents are within the limits of pharmacopeia.

Table 5: Characterization of Ions on EDM, EMZ & EBL Seed Powder

Chemical test	Observation	Inference		
		EDM	EBL	EMZ
Calcium To 5ml filtrate, add 1 drop dil. NH_4OH and saturated Ammonium Oxalate solution. With solution of NH_4CO_3	White ppt. White ppt.	+ve +ve	+ve +ve	+ve +ve
Magnesium Filter & separate while calcium oxalate ppt. obtained above. Heat and cool filtrate which with solution of Na phosphate in dilute ammonia solution	White crystalline ppt.	+ve	+ve	+ve
Sodium Flame test: prepare a thick paste of ash of drug with conc. HCl. Take a paste on platinum wire loop, introduce in burner flame.	Golden yellow flame	+ve	+ve	+ve
Potassium Flame test	Violet color flame	+ve	+ve	+ve
Iron To 5ml test solution add few drops 5% Ammonium thiocyanate	Blood red Color	+ve	+ve	+ve
Sulphate With lead acetate reagent	White ppt. soluble in NaOH	+ve	+ve	+ve
Phosphate To 5ml test solution prepared in HNO_3, add few drops of ammonium molybdate solution. Heat 10 min. cool.	Yellow crystalline ppt.	-ve	-ve	-ve
Chloride To about 5 to 7 ml filtrate, add 3to 5 ml lead acetate solution	White ppt. soluble in hot water	+ve	+ve	+ve
Carbonate With dil. Acid	Liberate CO_2	+ve	+ve	+ve
Nitrates Warmed with sulphuric acid & copper	Liberates red fumes	-ve	-ve	-ve

EDM- Extract of *Diospyros melonoxylon*, EBL- Extract of *Buchanania lanzan*
EMZ- Extract of *Manilkara zapota*

Table 6: Technological CHNS & Heavy Metal Characterization of EDM, EBL & EMZ

Polymer	C (%)	H (%)	N (%)	$W_{C/N}$	S (mg/kg)	Arsenic	Lead	Cadmium	Mercury
EDM	78.8	12.2	0.634	124.29	681	ND	ND	ND	ND
EBL	4.32	80.71	10.1	0.4277	1319	ND	ND	ND	ND
EMZ	80.9	10.1	1.58	51.202	512	ND	ND	ND	ND

EDM- Extract of *Diospyros melonoxylon*, EBL- Extract of *Buchanania lanzan*
EMZ- Extract of *Manilkara zapota*, ND= Not detectable

Mineral Elemental, CHNS and Heavy metal characterization

The extracts of all the seeds revealed the presence of Calcium, Magnesium, Sodium, Potassium, Iron, sulphate, chloride and Carbonates [Table 4]. The amount of C, H, N and S were observed and tabulated in table [Table 5]. The amount of heavy metals (Arsenic, lead, cadmium and mercury) are not detectable in all the seeds. Undesirable presence of heavy metals and aluminium may limit the use of plant material as food additives.

CONCLUSION

As there is no pharmacognostic/anatomical work and characterization of ions and heavy metals on record of these local plants of Chhattisgarh, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of these medicinally useful plants.

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Pharmacognostic Evaluation of *Alternanthera Sessilis (L.) R.Br.ex.DC*

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ABSTRACT

Alternanthera sessilis L. is a leafy vegetable used widely for its medicinal properties. The lack of documentation of medicinal plants have lead to the loss of quality in many important plants .The objective of the work was to study the pharmacognostic features of this plant. The study revealed the presence of calcium oxalate crystals in both stem and leaves. Extensively longer uniseriate trichome measuring 1227.4-1431.9 µm was observed in powder anaylsis. Macrosclerids and scalariform thickening were characteristic observation in the stem maceration. The vascular bundle showed inter fascicular cambial ring which is an anamolous secondary growth. The phytochemical studies on aqueous extracts of leaf showed positive result for Phenols, flavonoids, tannins and saponins. This study will help in standardizing and detection of adulterants.

Key words: Alternanthera sessilis , Pharmacognostic evaluation

INTRODUCTION

Alternanthera sessilis (L.) DC. (Sessile joy weed).It is a popular leafy vegetable Its leaves and young shoots are eaten as vegetable [1]or cooked in soup in Sri Lanka and also used as traditional medicine in China, Taiwan and India. *Alternanthera sessilis* (Amaranthaceae) is widespread throughout the tropics and subtropics and is an annual or perennial prostate herb with several spreading branches, bearing short petioled simple leaves and small white flowers, found through out the hotter part of India, ascending to an altitude of 1200m[2] in the Himalayas and even cultivated as a potherb.

A.sessilis is known as Matyakshika in Ayurvedic medicine[3]. It has been used in Indian traditional system of medicine since a long time in diseases due to vitiated blood, skin diseases and ulcers[4]. Its active principles, extracted in oil, were used to treat infected wounds and the herb also proved styptic in colitis; its nutritive values make the herb a potent tonic with a wide range of applications. Poultice of pounded fresh material is used in sprains, burns and eczema, carbuncle, erysipelas and acute conjunctivitis. A decoction is recommended as a herbal remedy to treat wounds, flatulence, nausea, vomiting, cough, bronchitis, diarrhea, dysentery and diabetes. Its roots can relieve inflamed wounds[5]. The leaves and shoots are boiled and drunk as an antihypertensive remedy[6].

It is also used as a cholagogue , galactogogue, abortifacient, febrifuge and to treat snakebites, dysentery, diarrhea, skin problems inflamed wounds and boils, and applied externally on acne and pimples[7-11]. In some parts of Bihar (India) the plant is used for hazy vision, night blindness, and post-natal complaints. *A. sessilis* has been reported to posses anti-microbial, molluscicidal, a moderate antimutagenic, antidiarrhoeal ,hepatoprotective, cytotoxic and antiviral activities.[12-13] The wound healing property of *Alternanthera sessilis* was reported by[14]. Degenerative and necrotic changes in the liver and kidney in Swiss mice, caused by oral administration of water extract of *A. sessilis* in high doses was revealed through histopathological test[15]. Previous phytochemical studies have reported the isolation of flavonols, triterpenoids, steroids and tannins; ß-sitosterol, stigmasterol, campesterol, lupeol being few of its important constituents[16]. The petroleum ether and benzene extracts inhibit the growth of some human and plant pathogenic bacteria[17]. Previous study on this plant showed that it has hepatoprotective activity[18] and potent nootropic activity[19].

Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plants parts to be potential source of medicinal substances. However a key obstacles which has hindered the acceptance of the alternative medicines in the developing countries, is the lack of documentation and stringent quality control. There

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is a need for documentation of research work carried out on traditional medicines with this back drop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. Hence pharmacognostic and phytochemical evaluation of *Alternanthera sessilis* is reported .

Materials and Methods

Fresh material was collected from the field and a voucher specimen was deposited in the Department herbarium collection.

Macroscopic and microscopic study

The macroscopic characters such as description of the plant, colour, odour, taste, nature, texture were studied for morphological investigation. For anatomical studies Free hand section of stem and leaves were taken, stained with safranin and mounted in glycerol and observed under light microscope and photographed. The quantitative microscopy was studied as per the procedure of[20-21]

Maceration

The stems of *Alternanthera sessilis* were cut into small piece, boiled in water and cooled material was repeatedly boiled to expel air and cooled for 3-5 times till the pieces settled down. Treated pieces of the plant were soaked in Jeffery's fluid (equal volume of 10% of nitric acid and 10% of chromic acid) for 24 hours at 30-40 ºC, decanted, washed and then stored in 50% alcohol. Pieces of macerated stem treated with aqueous safranin overnight , dehydrated through alcohol series (50%, 60%, 70%. 80%, 90%, 100%) for five minutes and passed through alcohol: xylol (1:1 ratio) series for five minutes and then the material was macerated and observed.

Histochemical test

The plant section was treated with various reagent such as wagners reagent (potassium iodide and iodine) for detection of alkaloid, orcinol in sulphuric acid for gums, Toluidine blue O for lignin, copper acetate test for Terpenoids, ferric chloride in 1N Hydrochloric acid for tannin, Sulphuric acid for crystals , methylene blue test for phenols.

Phytochemical Screening

The dried leaves were extracted with sterile distilled water. The behavior of powder with various chemical reagent and preliminary chemical tests was carried out according to the standard procedures described by Kokate[22] and Horborne[23].

Powder Analysis

The fine powder of *Alternanthera sessilis* was obtained by air drying the leaves. The dried leaves were powdered and sieved to obtain fine powder. The fluorescence analysis was done according to[24].

RESULTS AND DISCUSSION

Macroscopy

Alternanthera sessilis is a prostrate or procumbent, annual or perennial herb. The branches are raised from the root and are up to 50 cm long. They are often purplish in colour and glabrous. The leaves are fleshy, generally 1.3-3 centimetres long and 0.5-1 centimetres wide though the leaves are larger in wet habitats, linear-elliptic, oblong or obovate, apex rounded and base cuneate. The flowers are inconspicuous, white, borne in small, axillary heads; bracts are ovate or obovate and are 1 mm long. The bracteoles are shorter, persistent; subequal, ovate and acute. Utricleare cordi-form and are strongly compressed. The seeds are orbicular. The plant bears flowers and fruits throughout the year.

Microscopy

Microscopical studies are useful to establish the botanical identity for the valuable herbal drugs, which forms the basis for the identification and determination of adulterants. The anatomical study of the stem revealed a single layered epidermis. There were 2-3 layers of collenchyma cells .The cortex was made up of 7-8 layers parenchymatous cells.The material under investigation was a tender stem and hence does not exhibit secondary growth. However anamolous growth of the cambium was evident. The interfasicular cambium forms a cambial ring. Intrafasicular cambium was also evident Vascular bundle consisted of xylem and phloem. Characteristic rosette crystals were present scattered in the cortex and in the pith. These were calcium oxalate crystals.

The leaf was differentiated into upper epidermis and lower epidermis. Mesophyll was differentiated into palisade and spongy parenchyma. spongy parenchyma cell were compactly arranged with no intercellular spaces. In upper epidermis the palisade cell were arranged in three layers. Vascular bundles contain xylem and phloem. Xylem is surrounded by phloem. It contains no distinct bundle sheath and dense starch accumulation in mesophyll cells. Mesophyll cell also harbored calcium oxalate crystals in a scattered manner (Plate-1).

Quantitative Microscopy

Quantitative microscopic data are found to be constant for a species. These values are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant. The study of the leaf constants showed that the average stomatal number is 82 and the stomatal index was 113.8%. The vein islets number and vein termination numbers were 33.5-37 and 28-31/sqmm respectively. The microscopic linear measurement of the trichomes showed that the length of the trichome was 1227.4-1431.9 μm and the width was around 122.7-204.6 μm (Table-1).

Maceration

The macerated stem of the plant showed various structure. *Alternanthera sessils* showed scalariform thickening in the trachieds with macrosclerids (Plate-1) .

Powder Analysis

The powder characters of a drug are mainly used in the identification of the drug in the powder from. The leaf powder was pale green in colour , fragrant , tasteless and papery in texture. The microscopical examination the powder showed anomocytic stomata,unicellular trichome , epidermal cells and calcium oxalate crystals.(Plate-1)

Histochemical test

The Histochemical studies showed the blue, bluish green or brown pigmentation for the presence of tannin and lignin in *Alternanthera sessilis*. Absence of Brisk effervescence with sulphuric acid revealed the existence of calcium oxalate crystals.

Table 1: Quantitative microscopy of *Alternanthera sessilis*

S.No	Quantitative characters	Numbers
1	Stomatal number	82
2	Stomatal Index	113.8%
3	Vein–islet number	33.5-37/Sq.mm
4	Vein termination number	28-31/Sq.mm
5	Trichome length	1227.4-1431.9 µm
6	Trichome breadth	122.7-204.6 µm

Phytochemical tests

The aqueous extract revelaed the presence of Phenols, flavonoids, tannins, saponins were present and remaining phytochemicals were absent (Table-2).

Fluorescence analysis

The fluorescence analysis of powdered leaves was studied in both UV and day light. The powder showed fluorescent green colour with UV light at 365 nm, which indicates the presence of chormophore in the drug (Table-3).

CONCLUSIONS

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy and microscopy parameters. As there is no record on pharmacognostical work on

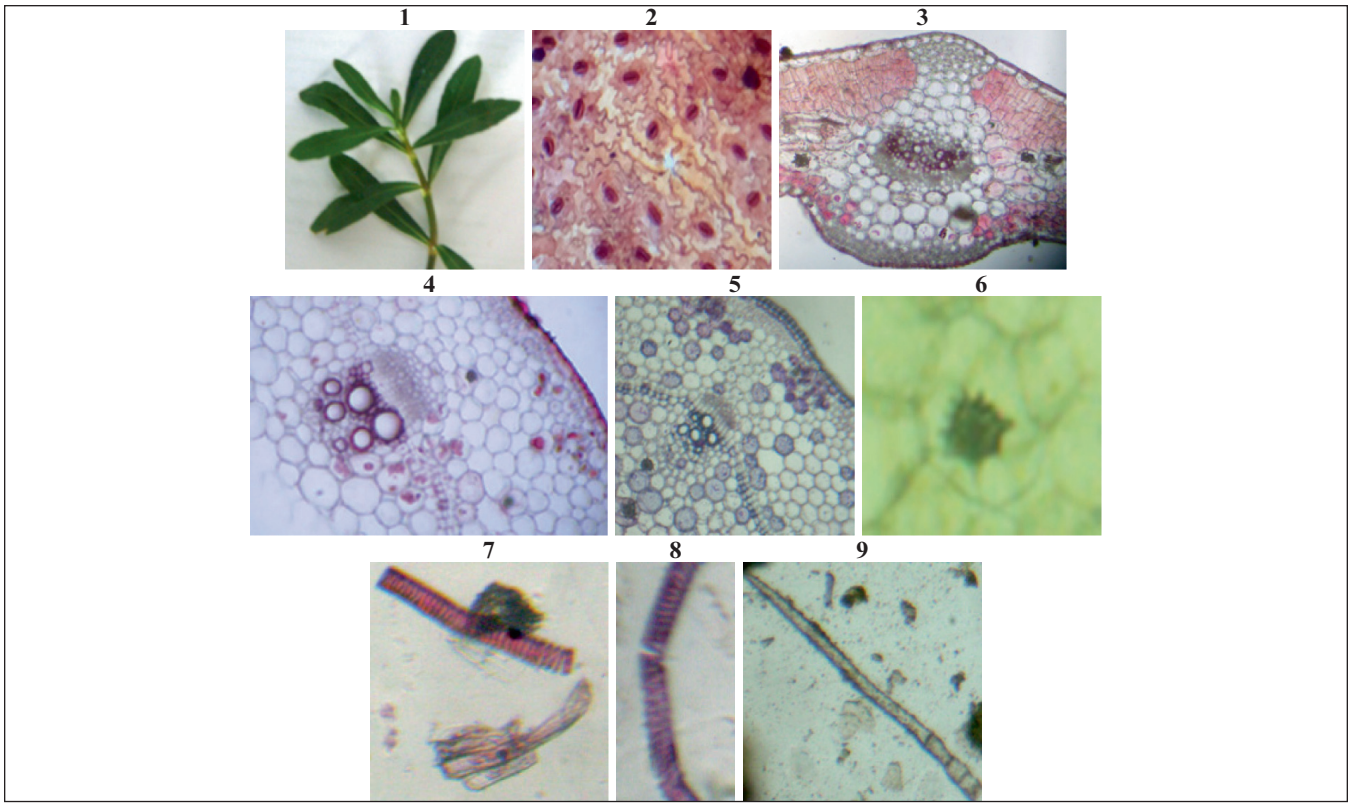

Plate 1: Pharmacognistic features of *Alternanthera Sessilis*

1-Habit of *Alternanthera sessilis* 2-Epidermal peeling - Anomocytic Stomata 3-C.S of leaf showing calcium oxalate crystals 4-T.S of stem stained with safranin 5-Histochemical test of for Phenols with Toludene blue O 6-Rosette calcium oxalate crystal 7-macrosclerids 8-Scalariform thickening in xylem tracheids 9-Uniseriate trichome. Magnification -10 X

Alternanthera sessilis Linn. The present work is undertaken to produce some pharmacognostical standards. The above studies provide information with respect to their identification and chemical constituent characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of ayurvedic pharmacopoeia. It will also determine therapeutic diagnostic tools for the scientistics who are keen and sincere to evaluate the herbal medicine of indigenous resources. The present pharmacognostic studies on *Alternanthera sessils* will be of great importance in detecting adulterant in these herbal drugs.

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Table 2: Phytochemical test of aqueous extract of *Alternanthera sessilis*

S.No	Aqueous Extract	Phytochemical test
1	Alkaloids	–
2	Phenols	+
3	Flavonoids	+
4	Tannins	+
5	Terpenoids	–
6	Gums	–
7	Resins	–
8	Steroids	–
9	Saponins	+
10	Glycosides	–
11	Anthroquinone	–
12	Phlobatanmin	–

Table 3: Powder analysis under white and UV light

Dry powder	Ordinary	Hydrochloric acid	Sodium hydroxide & methanol	Sodium hydroxide & water	Nitric acid	Sulphuric acid
White light	Dull green	Green	Dark green	Yellowish green	Brown orange	Yellow green
UV Light 365nm	Fluorescent green	Green	Dark brown	Brown	Brown	Green
UV Light 265nm	Fluorescent yellow	Fluorescent yellow	Fluorescent yellow	Fluorescent yellow	Fluorescent yellow	Brown

Pharmacognostic studies on the flowers of *Acacia nilotica* Linn

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ABSTRACT

Acacia nilotica (Linn) family Mimosaceae commonly known as Karuvalem is used in Traditional System of Medicine for healing various diseases. It is used in the treatment of throat troubles, stomatitis, chronic dysentery, diarrhoea and in conjunctivitis. In the present investigation an attempt has been made for the pharmacognostical standardization of *Acacia nilotica* flowers. The pharmacognostical evaluation comprises of morpho-anatomy, histochemistry and physical constants such as ash, loss on drying and extractive values of *A.nilotica* flowers. The flowers extracts were subjected to preliminary phytochemical screening. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulation containing A.nilotica.

Keywords: *Acacia nilotica*; Pharmacognostical evaluation; Physico-chemical data.

INTRODUCTION

Plants are the essential and integral part in complementary and alternative medicine.The ability for the formation of secondary metabolite like flavonoids, alkaloids, steroids and phenolic substances makes the plants to be used to restore health and heal many diseases. Natural products of plant and animal origin offer vast resources of newer medicinal agent with potential in clinical use.

Acacia, a leguminous genus belonging to the family of Mimosaceae, comprises approximately, 1200 species that are dispersed widely in tropical and subtropical regions of Australia, south America, Asia and Africa[1-2]. Many of these species are important for fuelwood, timbers, shelter belts and soil improvement[3]. It has been used extensively for the treatment of various diseases eg. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma[4]. It also serves as a source of various products, including polyphenols[5,6]. The role of these natural products to the plant itself is not well understood, but for the human kind they can be of prime importance. Therefore, the bioprospection of naturally occurring polyphenolic compounds having ability to provide protection against certain types of mutagens and carcinogens is of great importance[7]. *A.nilotica* contains gallic acid, m-digallic acid, (+)-catechin, chlorogenic acid, catechin-5-galloyl ester[8]. *A.nilotica* has anticancer and antimutagenic, anti-inflammatory, antiplasmodial[9], antidiarrhoeal[10], antihypertensive, antiplatelet aggregatory, molluscicidal, antifungal, antimicrobial activity, inhibitory activity against Hepatitis C and HIV-I[11]. Eventhough the plant is rich in bio-active constituents and potential therapeutic activities there is a lacuna in the pharmacognostical standardization on the flowers of *A.nilotica* Linn. So the present investigation was aimed at evaluating the pharmacognostical features and phytochemical analysis for authentication and identification of the plant and also to evaluate the exact extract responsible for the biological activity.

Materials and Methods

The fresh flowers of *A.nilotica* Linn., were collected from Thanjavur Tamilnadu, India, (August 2010), which was identified by Taxonomist, and authenticated by Prof.P.Jayaraman, Botanist, Director, Plant anatomy Research Centre, Tambaram. A voucher speciemen no were deposited in the Department of CSMDRIA ,Arumbakkam,Chennai for the future reference. The fresh flowers were collected and fixed immediately using FAA (Formalin: Acetic acid: Ethyl alcohol) as fixative agent for anatomical studies. The materials were cut into small pieces before fixing.

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Pharmacognostical studies

Macroscopic

Morphological studies were done by using simple microscope to determine the shape, nature, texture, colour, odour and taste of the flower.

Microscopic

For anatomical studies customary technique of microtomy was followed[12]. Paraffin section of 10µm thick were stained with Toludine blue. Photomicrography were taken with Nikon lab photo-microscopic unit. The chemomicroscopy were carried out according to the methods outlined by[13-15].

Physicochemical studies

The ash values, extractive values and loss on drying at 105ºC were performed according to the official methods prescribed in Indian Pharmacopoeia[16] and the WHO guidelines on quality control methods for medicinal plant materials[17].

Preliminary phytochemical analysis

For preliminary phytochemical analysis, extracts was prepared by weighing 1kg of the dried powdered flowers subjecting it to hot successive continous extraction with different solvents in the order of increasing polarity namely petroleum ether, chloroform, ethyl acetate, methanol and finally with aqueous. The extracts were filtered separately, concentrated and the solvent was removed by rotary evaporator. The extracts were dried over desiccators and the residues were weighed. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods[18, 19].

Results and Discussion

Macroscopy

The flowers are yellow in colour. The sepals and petals are five each, stamens are numerous. The pods are moniliform, white and tomentose.

Microscopy

Microscopical studies are usefull to establish the botanical identity for the valuable herbal drugs.

Longitudinal section view

In longitudinal section, the inflorence has a peduncle, which dilates into hemispherical thalamus bearing numerous dense flowers. At the base of the thalamus is a ring of thick densely staining nectariferous gland (Fig 1). The flowers are sessile and are borne in the axil of a bract (Fig.2). The flower has gamosepalus calyx and gamopetalous corolla. The sepals are thin at the base and thick at the apex. The petals are uniform in thickness from the base to the tip (Fig 3). All the parts of the flower receive vascular strands from the surface of the thalamus.The prominent nectariferous gland and thick with slightly concave top portion. Vascular strands enter the nectary before extending to the flowers. The cells of the nectar are darkly stained (Fig 1).

Transverse section view

In transverse sectioned view, the flowers appear elliptical or circular. Each flower has a short, thick bract, thin outer cylinder of calyx and inner cylinder of corolla (Fig 4). Within the corolla tube occur sections of staminal filaments and anthers (Fig 5).The calyx tube consists of five thickened portions alternating with thin portion. The thick portion is about 6 layered including the inner and outer epidermal layers (Fig 6) and is 70µm in thickness. The thin portion is just three layered. The corolla tube also varies in thickness along five portions of the circumference. It consists of an epidermal layer of oblong thick walled cells and inner three or four layers of compact parenchyma cells (Fig 7). Anthers are dithecous and four celled. The anther wall consists of an outer epidermis, inner endothecium with spiral thickenings and inner epidermis which is mostly disintegrated during microsporogenesis. The pollen grains are large with prominent reticulate markings of the exine (Fig 8).

Powder microscopic observations

The powder character of a flowers are mainly used for the identification of drug in the powder form. The powder was yellow in colour with strong and characteristic in taste. On microscopical examination the powder showed the following inclusion;

Epidermal trichomes (Fig 9)

Unicellular, unbranched trichomes are occasionally seen in the powder. They have very thick reticulate lignified walls and narrow lumen.The trichomes are 250µm long and 10µm thick.

Stamens (Fig 10)

Isolated stamens and anthers are common in powder. The filaments are thick and cork like; they are multicellular, multiseriate with vertically oblong cells. The filament is 400µm long.There are also anthers attached to the filament and detached anthers. The anthers are squarish in side view, dark and measure 120µm thick and 150µm wide .

Pollen grains (Fig 11)

Pollen grains are seen scattered in the powder. They are spheroidal in shape with ridged and furrowed surface. The exine consists of deeply marked reticulate and culpturing. The pollen is 40µm in diameter it is darkly coloured.

Physicochemical standardization of flowers

The air dried, powdered plant materials were subjected for determination of various physicochemical standardization parameters as per the method described in[21].

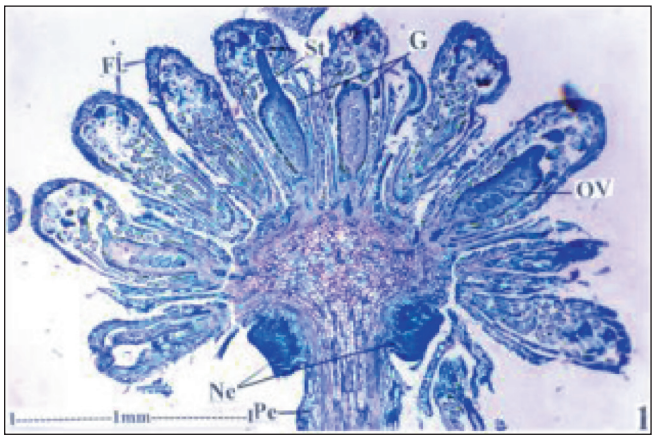

Figure 1: Longitudinal section of Acacia nilotica inflorescence. Abbreviations: Ne-Nectar gland; Pe-Peduncle; Fi-Filament; St-style; G-Gynoecium; Ov-Ovary

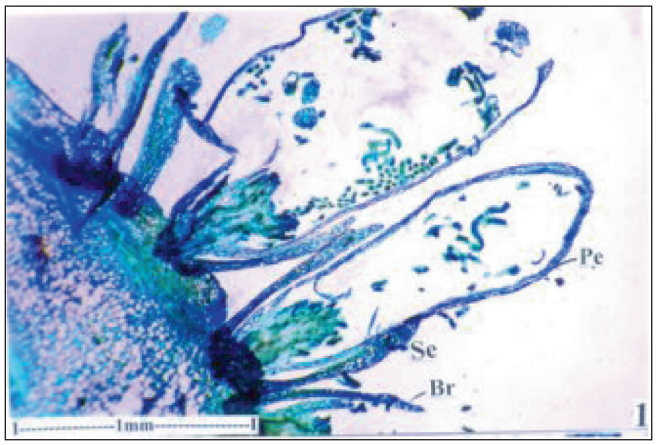

Figure 2: Longitudinal section of flower under 4X Power. Abbreviations: Pe-Petal lobe; Se-Sepal; Br-Bract

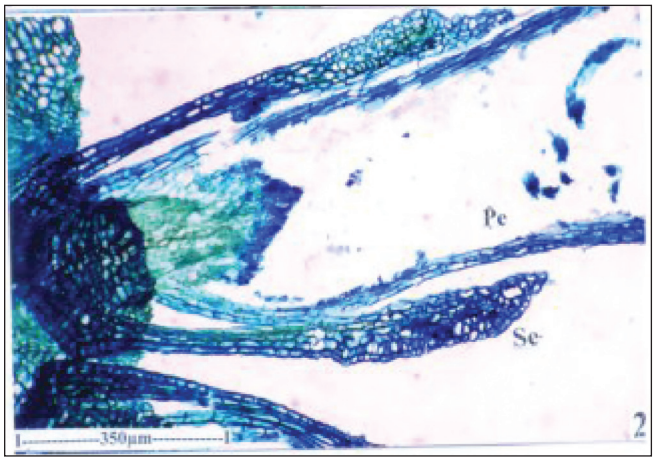

Figure 3: Longitudinal section of Sepal. Abbreviation: Pe-Petal lobe; Se-Sepal

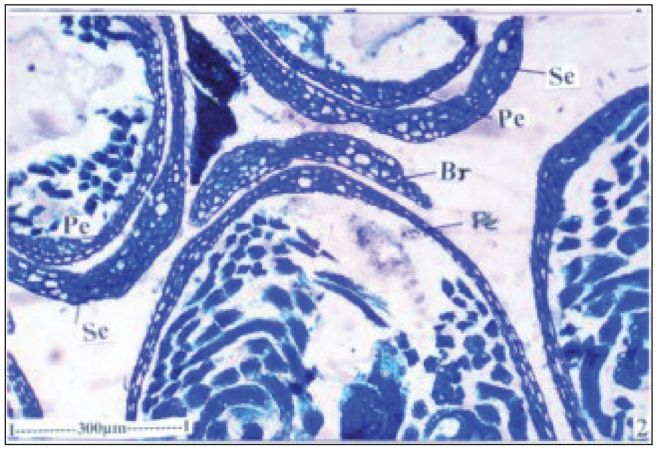

Figure 4: Transverse section of Flower under 10X. Abbreviation: Pe-Petal lobe; Se-Sepal, Br-Bract

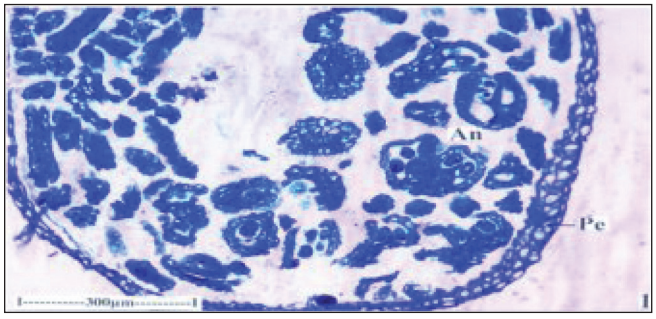

Figure 5: Transverse section of Corrola. Abbreviation: Pe-Petal lobe; An-Anther

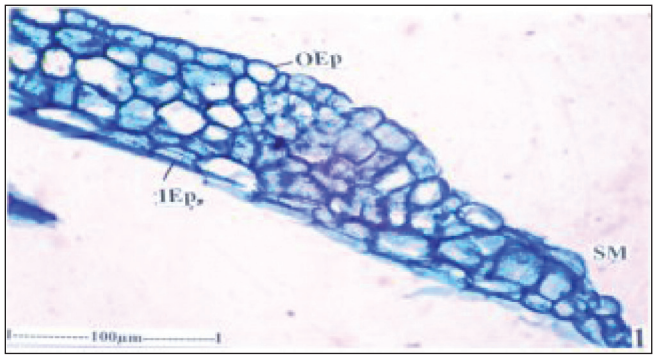

Figure 6: Epidermal layer. Abbreviation: IEP-Inner epidermis; SM-Sepal Margin

Determination of ash values

The percentage of loss on drying, total ash values, water soluble ash and acid insoluble ash were determined. The results obtained were; loss on drying (6.23± 0.19 %), total ash (4.48 ± 0.38%), water soluble ash (1.69 ± 0.39%), and acid insoluble ash (0.56 ± 0.10%) respectively. The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crude drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and it is an important parameter for the evaluation of crude drugs. In certain drug, the percentage

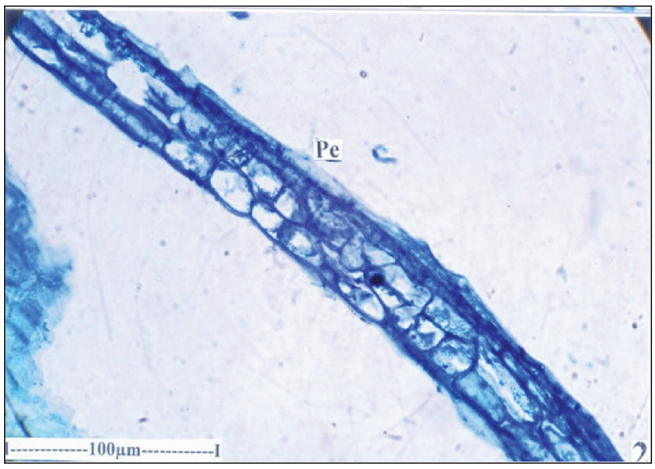

Figure 7: Parenchyma cells.

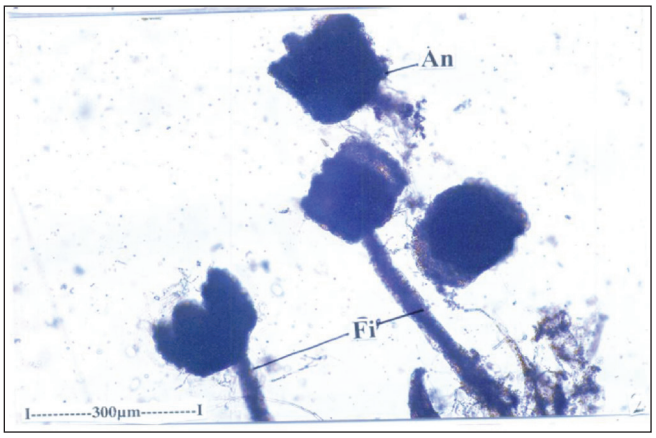

Figure 10: Stamens.
Abbreviations: Fi-Filament; An-Anther

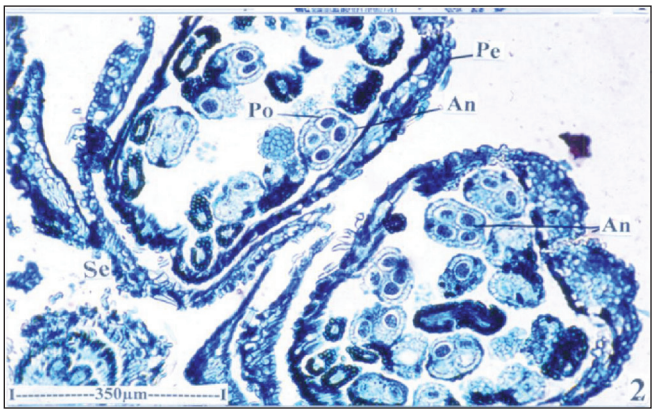

Figure 8: Transverse section of Anther.
Abbreviation: Pe-Petal lobe; PO-Pollen; An-Anther

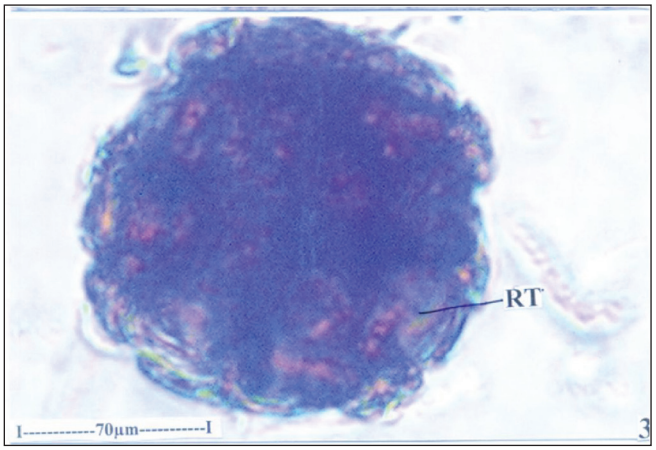

Figure 11: Pollen.
Abbreviations: RT-Reticulate

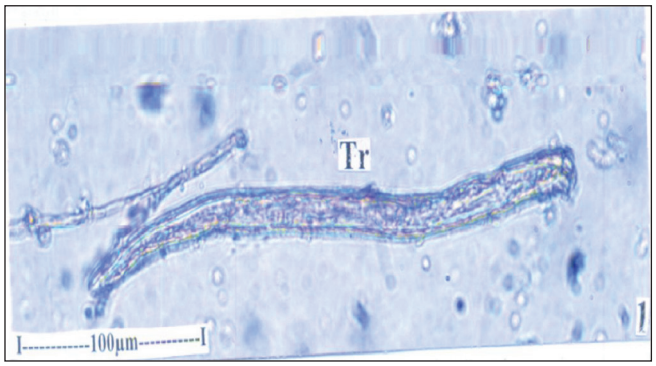

Figure 9: Epidermal Trichomes.

Table 1: Data showing the Physico-chemical standard vaues of *Acacia nilotica* Linn

Parameters	Values
Total ash	4.48 ± 0.38
Acid insoluble ash	0.56 ± 0.10
Water soluble ash	1.69 ± 0.31
Loss on drying at 105°C	6.23 ± 0.19
Extractive value	
(i) Petroleum ether	0.569 ± 0.23
(ii) Chloroform	0.46 ± 0.25
(iii) Ethyl acetate	1.72 ± 0.32
(iv) Methanol	13.39 ± 0.12
(v) Aqueous	12.40 ± 0.20

The results are the mean of five estimation ± standard error

variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time posses a character that will raise the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value**,** acid insoluble ash value, water-soluble ash values were determined as per WHO guide lines. The results and observation are presented in Table 1.

Extractive value

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. The extractions of any crude drug with a particular solvent yield a solution containing

Table 2. Data showing Qualitative analysis of the phytochemicals of the *Acacia nilotica* Linn.

Extracts	Sterols	Terpenoids	Carbohydrates	Flavonoids	Alkaloids	Glycosides	Saponins	Tannins
Petroleum ether extract	+	+	–	–	–	–	–	+
Chloroform extract	+	+	–	–	+	–	–	+
Ethyl acetate extract	–	–	+	+	–	–	–	+
Methanolic extract	+	+	+	+	+	–	+	+
Aqueous extract	–	–	+	+	-	–	+	+

+ Presence of constituent ; – Absence of constituent

different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and solvent use. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not). The mean value obtained for each of these parameters was found to be consistent with minimum standard deviation.

Preliminary phytochemical analysis

The various extracts were subjected to preliminary phytoconstituents analysis for their presence or absence of the constituents. The results are shown in Table-2. The plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects. Secondary metabolites are the compounds which are responsible for imparting therapeutic effects. The phytochemical screening showed that the flowers were rich in saponin, flavonoids, tannins and terpenoids. Steroids were found to be present in the flowers. It has been found that this plant contained steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones[20]. Therapeutic activity may be due to these compounds.

CONCLUSION

In the present days of modernization, Ayurveda no longer can afford to remain confined to use of conventional norms of medication. It has to accept the new challenges and be prepared to answer the queries of the modern man about the quality and efficacy of the herbal drugs administered to him and also how they are collected, processed, preserved and used. The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful in standardization of herbal drugs of folk medicinal practice of present era and enrichment of Ayurvedic pharmacopoeia.

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Pharmacognostic profile of root of *Cryptolepis sanguinolenta* (lindl.) Schlechter

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ABSTRACT

Medicinal plants are traditionally found to be useful for many ailments. The present study highlights the pharmacognostical as well as phytochemical studies including parameters such as macroscopic, microscopic characters, physicochemical evaluation, chemomicroscopy and preliminary phytochemical studies of the root of *Cryptolepis sanguinolenta*. The morphological studies shows a root light to medium brown in color with hard and brittle texture, prisms of calcium oxalate crystals, sclereids and parenchyma cells. The transverse section showed parenchyma cells, vascular cambium, ray lines and phelloderm. Physico-chemical standards and their percentage values w/w were found to be: total ash (14.02 ± 0.12), acid insoluble ash (5.08 ± 0.18, %), water soluble ash (4.02 ± 0.27 %), sulphated ash (4.26 ± 0.11 %), alcohol soluble extractive (6.20 ± 0.45 %), water soluble extractive (28.40 ± 0.75 %) and moisture content (6.80 ± 0.25 %). Chemomicroscopical investigation revealed presence of lignin, tannin, oils, cellulose and calcium oxalate. Phytochemical analysis of the root revealed the presence of carbohydrates, alkaloids, glycosides, saponins, resins, proteins, steroids and terpenoids. These findings will help in identification, standardization of the root of *Cryptolepis sanguinolenta* (Lindl.) Schlechter and also distinguish it from its adulterants.

Keywords: *Cryptolepis sanguinolenta*, chemomicroscopic analysis, phytochemical analysis, physiccochemical standards.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Throughout history, herbs had been used by all cultures as sources of medicine. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter and medicine. The use of plants as medicine is older than recorded history. Traditional and folklore medicine which was bequeathed from generation to generation is rich in domestic recipes and communal practice. About 1400 herbal preparations are widely used according to a recent survey in Member States of the European Union[1].

Cryptolepis sanguinolenta (Lindl.) Schlechter (Fam: Asclepiadaceae) the plant of interest is a shrub that grows in the rainforest and distributed throughout the West Coast of Africa. It is known as yellow dye root, *Akpaoku* (Igbo), *Gangamau* (Hausa), *Delboi* (Fulani).It is a twining, scrambling shrub, with characteristic thin stems and tuberous root stock. The leaves are opposite, thinly herbaceous, elliptic-oblong to ovate or lanceolate in shape up to 7 cm long and 3 cm wide. The margin is entire, the apex is curved and acuminate and the base is symmetrical and obtuse or rounded. The midrib projects prominently on the lower side and is pinnately nerved. The dried leaves have a slight bitter taste. It is used in hypertension, microbial infections, fever and stomach ache[2]. Clinical studies have shown that extract of the plant produced cures in patients with the concomitant elimination of parasitaemia in the blood[2]. The principal constituent of *Cryptolepis sanguinolenta* (Lindl) Schlechter is the indoloquinoline alkaloid, cryptolepine which occurs at a yield of 0.52 % in the roots, 0.48 % in the stem and 1.03 % in the leaves. The compound occurs with related bases and their derivatives. Cryptolepine has been found to produce antihyperglycemic and cytotoxic effects through GC-rich DNA sequence intercalation that provides basis for the design of new anticancer drug[3]. Another indolequinoline alkaloid quindoline synthesized from cryptolepine[4]. Dwuma –Badu *et al*[5] reported the isolation of quindoline from the

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roots of *Cryptolepis sanguinolenta*. Three new indole alkaloids hydroxycryptolepine, cryptoheptine and cryptoquindoline have been isolated and structurally elucidated as well[6]. Recently, a tetracyclic alkaloid, isocryptolepine has been isolated from the roots of *Cryptolepis sanguinolenta*[7]. Neocryptolepine and biscryptolepine (11-cryptolepin-11-y1)-cryptolepine), were isolated from the root bark of this plant and their structures elucidated[8, 9]. This study aims at establishing the macroscopic, microscopic, chemomicroscopic and physicochemical profiles of the root of *Cryptolepis sanguinolenta* that would be useful in preparing a monograph for identification of the plant.

MATERIALS AND METHODS

Collection of plant materials

The roots of *Cryptolepis sanguinolenta* were collected in February 2010 from Nsukka, Enugu State, Nigeria. It was identified and authenticated by Mr. A. Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development (INTERCEDD) Nsukka, Enugu State and a Voucher Specimen (UN/PCOG/010/401) deposited in the Herbarium of Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka.

Plant sample preparation

Fresh roots were collected, washed and excess water allowed to drain off. Representative samples were kept for examination while the rest were dried completely. They were pulverized and the powdered sample stored in airtight container for use in the microscopic, chemomicroscopic and phytochemical studies. Transverse sections were cut from the representative sample using sledge microtone. The sections were preserved in 70 % ethanol until needed for studies.

Macroscopical Examination

The macroscopical features of the fresh root were examined using the methods described by Evans[10].

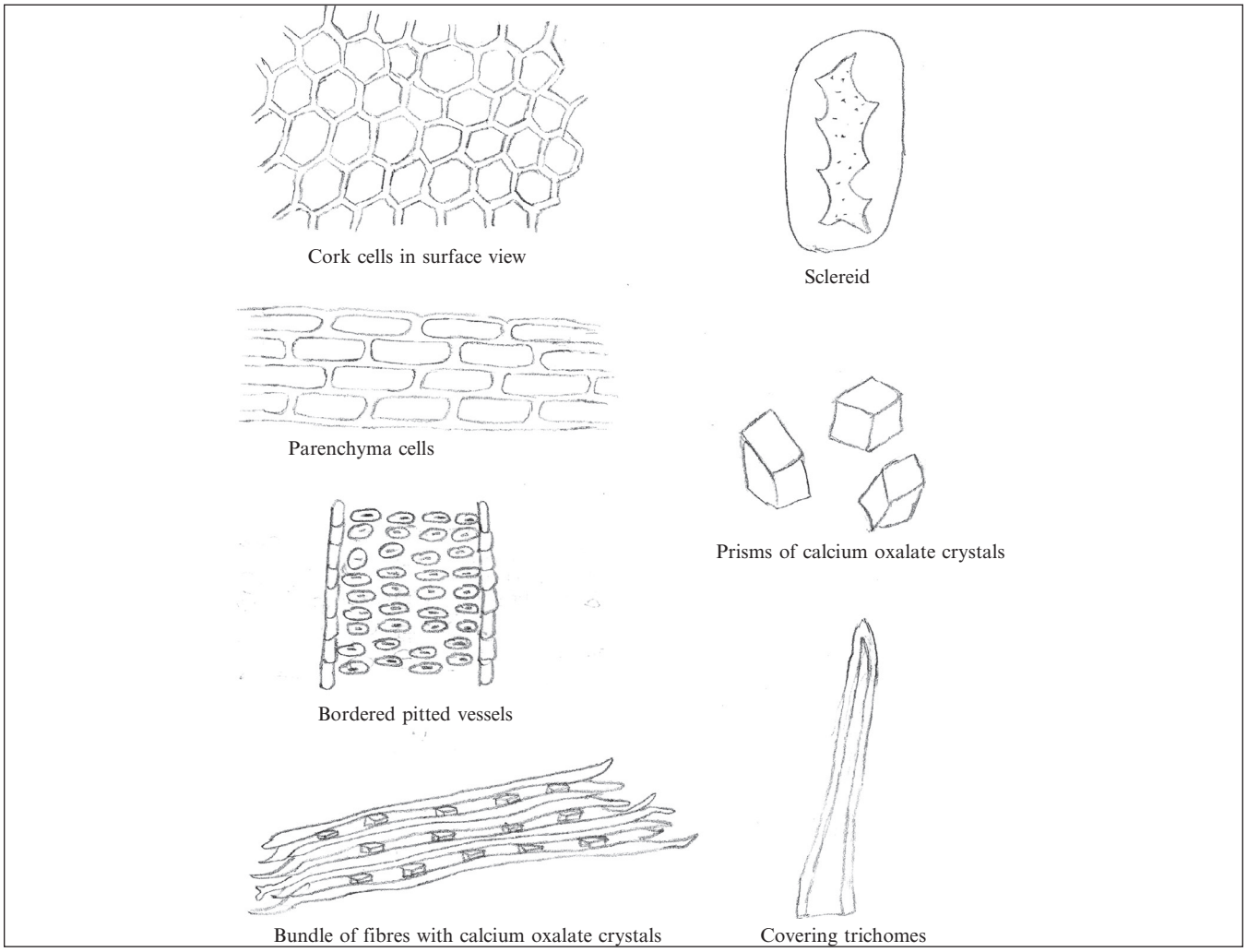
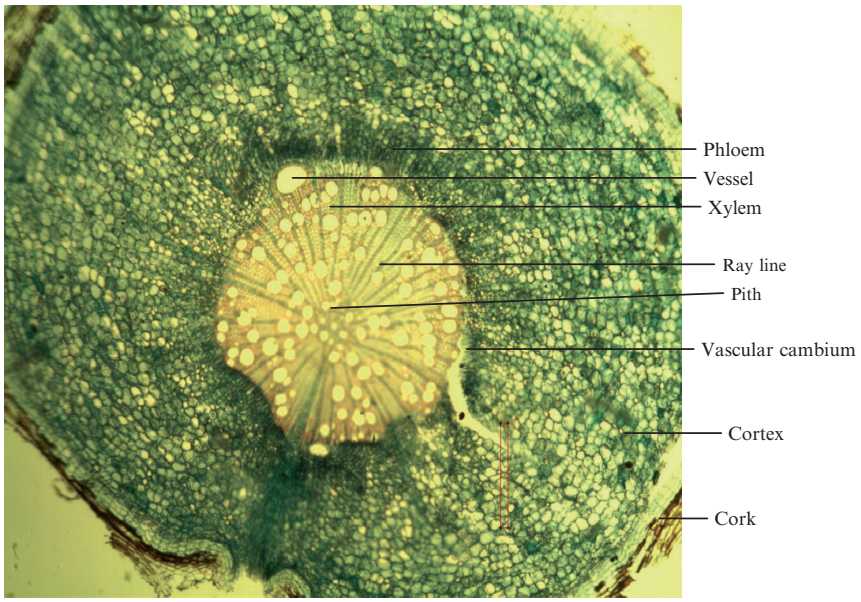


Figure 1: Microscopical features of root of *Cryptolepis sanquinolenta*



Figrue 2: Transverse section of the root of *Cryptolepis sanguinolenta*

Microscopical examination

Anatomical sections of the fresh root and powdered samples were prepared for the microscopic studies. The staining was done using standard laboratory methods[10 - 11].

Phytochemical studies

The preliminary phytochemical screening of the root powder was performed following standard qualitative chemical tests[10, 12] in other to detect the presence or absence of major secondary plant metabolites of pharmacognostic importance. The classes of phytoconstituents tested include; alkaloids, tannins, flavonoids, saponins, glycosides, proteins, fats and oils, steroids and carbohydrates.

Determination of some physicochemical standards

The water extractive and alcohol extractive, total ash, acid insoluble ash, water soluble ash and sulphated ash were determined as described by Evans[10]. The moisture content of the root powder was determined by loss on drying method[13].

Chemomicroscopic examination

Chemomicroscopical 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[10].

RESULT

Macroscopical examination

The root surface of *Cryptolepis sanguinolenta* is light to medium brown in colour. It is hard and brittle, with longitudinal ridges apparent in the dried samples. The roots are branched with little or no rootlets. The root breaks with a short fracture exposing a smooth surface which is yellow in colour and has a bitter taste.

Microscopic examination

The microscopical analysis of the root revealed the following features: cork with thin walled cells, polygonal and elongated in surface view, sclereids thickened with branched pits showing distinct strations. A unicellular covering trichomes, bundle of fibres with calcium oxalate crystals and fibres with bordered pitted vessels. There were also parenchyma cells.

Phytochemical studies

Phytochemical screening of the root powder revealed the presence of alkaloids, reducing sugars, flavonoids, saponins, glycosides, proteins, steroids, terpenoids and carbohydrates (Table 1).

Physicochemical standards

The water extractive and alcohol extractive, total ash, acid insoluble ash, water soluble ash and sulphated ash were shown in Table 2.

Chemo-microscopical Examination

The chemomicroscopy of *Cryptolepis sanguinolenta* revealed the presence of cellulose, starch, suberized wall, fibres, secretory cells and ducts and calcium oxalate crystals (Table 3).

DISCUSSION

The identification and evaluation of the root of *Cryptolepis sanguinolenta* have been carried out and the various

characteristics and features associated with it duely determined by the various analysis and tests performed. The macroscopic examination reveals the physical appearance of the root, which can be seen with the naked eyes. This however gives an idea of the part and cannot be relied solely for the identification of the root of the plant. The microscopic examination gives hints about the characteristic features that could be found in different morphological parts of plants. These features and their arrangements are not always the same in all morphological parts. The presence of abundant prisms of calcium oxalate which indicates the presence of the calcium salt of oxalic acid that is present usually at about 1.0 % in plants[10], the covering trichomes can be used as identifying characters of the plant. The results obtained for ash values, which are of tremendous importance in quality control is used to detect foreign organic matter and detection of adulteration of sand or earth[14] were within the British Pharmacopoeia (BP) specification. The ash values obtained were adequate within the limits of experimental error since the total ash, acid-insoluble, water soluble ash and sulphated ash were determined were within the BP specification. The extractive values are moderate. The moisture content of the crude drug is not high (fall within the limit of the general requirement 8 – 14 %), indicating less probability of microbial degradation. Excess moisture in crude drug may lead to the breakdown of important constituent and the growth of microorganisms especially during storage of drug[15].

Table 1: Phytochemical analysis of the root of *Cryptolepis sanguinolenta*

Constituent	Inference
Carbohydrates	++
Reducing sugars	+
Alkaloids	+++
Glycosides	+
Saponins	+
Tannins	+
Flavonoids	+
Resins	+
Proteins	+
Fats and Oils	–
Steroids	+
Terpenoids	++
Acidic compounds	–

Key: + = slightly present; ++ = moderately present; +++ = highly present; – = Absent

Table 2: Qualitative evaluation of the root of *Cryptolepis sanguinolenta*

Parameter	Value % (w/w)
Total ash	14.02 ± 0.12
Acid insoluble ash	5.08 ± 0.18
Water soluble ash	4.02 ± 0.27
Sulphated ash	4.26 ± 0.11
Alcohol soluble extractive	6.20 ± 0.45
Water soluble extractive	28.40 ± 0.75
Moisture content	6.80 ± 0.25

Values are Mean ± SEM, n = 3.

CONCLUSION

The results obtained from this study can serve as diagnostic parameters for proper identification as well as preparation of a monograph on *Cryptolepis sanguinolenta*

ACKNOWLEDGEMENT

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

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Table 3: Chemomicroscopy of the root of Cryptolepis sanguinolenta

Test reagent	Observation	Inference
Iodinated zinc chloride solution	Blue colour observe d on epidermal cells	Cellulose present
Iodinated zinc chloride solution	Blue-black coloration observed in the xylem vessels	Starch present
Iodinated zinc chloride solution	Brown coloration observed	Suberized wall present
Phloroglucinol + Conc. HCl	No red colour observed in xylem vessels and phloem fibres	Lignin absent
80% H_2SO4	Bright crystals of calcium oxalate dissolved	Calcium oxalate crystals present
Sudan III	Pink-red coloration observed	]Fibres present
Ferric chloride solution	No greenish colour in parenchyma cells	Tannins absent
Sudan IV	No pink colour observed	Lipid absent
Picric acid solution	Yellow coloration observed	Secretory cells and ducts present

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Pharmacognostic evaluation of Nelsonia canescens. (Lam) Spreng. (Acanthaceae) Root

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Pharmacognostic evaluation of *Nelsonia canescens*. (Lam) Spreng. (Acanthaceae) Root

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ABSTRACT

Introduction: *Nelsonia canescens* (Acanthaceae), commonly used in name of 'Rasna (Bodo Rasna)' in Orissa, its root is being recommended in cases of pain and inflammation. Ethnic people use this plant very commonly. Review of the literature did not reveal much work on this plant, but ethnomedically root is said to be good anti inflammatory properties. Hence, it was thought worth to study the root of this plant. **Method:** The present investigation deals with macroscopic, microscopic study of the *Nelsonia canescens* root including its powder characteristics following standard procedures. **Results:** Chief microscopical characters include parenchymatous cortical zone, inner wall of the cell get detached and embedded in different form in cell cavities, medullary rays arising from the primary bundles, uni to tri seriate and getting wider towards periphery. **Conclusion:** Such a study would serve as a useful gauge in standardization of the root material and ensuring quality of the formulations.

Key words: Pharmacognosy, *Nelsonia canescens*.(Lam) Spreng, Rasna, Anti inflammatory, Gandhamardan hill ranges, Orissa

INTRODUCTION

Nelsonia canescens found throughout India except in the western desert areas and ascending to 1200m, in the Himalaya[1]. It is a diffused herb with thick fleshy roots; shoots densely villous. Leaves elliptic, obovate-oblong, subentire to crenulated, obtuse, pubescent, hairy or villous both sides, lower large and sometimes 20 × 7.5 cm or more or sometime absent, upper few or one pair of smaller, sub sessile, 1.2-5 cm long; petiole of lower leaves up to 4 cm. Flowers bluish purple, in ovate or cylindrical, glandular and villous spikes, 1.8-6.2 cm long; bracts ovate, 6.2 mm long, obtuse, glandular. Lowest calyx lobes broadest. Corolla 3-6.2 mm long, throat and palate with curled villi; upper lip erects of 2 lanceolate or oblong lobes, lower spreading with 3 sub equal rounded lobes. Capsule narrowly conical, 6.2 mm long. Seeds 5-1 in the lower part of each cell, closely minutely granulate[2].

Nelsonia canescens (Lam.) Spreng. is the plant from Acanthaceae family. This plant is used in traditional folk medicine in Africa and India[1]. Systemic application of *Nelsonia canescens* (Lam.) Spreng. is common practice for treatment of inflammation[1]. Hence it was considered that investigating this plant for these medicinal properties might give scientific authentication to the traditional claims. *N. canescens* root is used as Rasna in Orissa[3] (It is also found in the Gujarat[4,5], Assam[6], Madras[7]), but no pharmacognostical work has been done yet on this plant. Rasna was one plant and very well known up to 6th B.C. but the vaidyas of different parts of India relied upon the later writers and commentators more and lost touch with the neighbouring flora. Commentators have confounded confusion more[8]. But this deplorable state of affairs now should be remedied. But local pride and jealousy come in their way to grasp this situation. Every vaidya think that he used as Rasna is right genuine and there is no end to this[8]. Recent studies shows that Roots of *Nelsonia canescens* have mild to moderate anti-inflammatory against acute inflammation. It also possesses marked analgesic activity at higher dose. It is having significant anti-inflammatory against chronic inflammation. Hence it can be preferred in the treatment of pain and inflammation[9].

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MATERIALS AND METHODS

Collection and authentication

In the month of January, *Nelsonia canescens* (Lam) Spreng was collected from its natural habitat of Gandhamardana

Figure 1: Fresh plant of Nelsonia *cannescens*

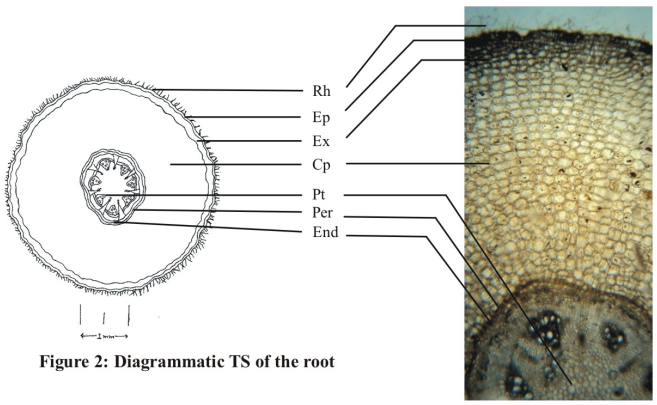


Figure 2: Diagrammatic TS of the root

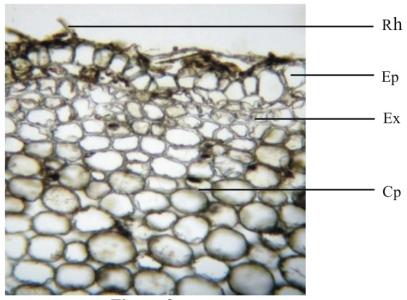


Figure 3

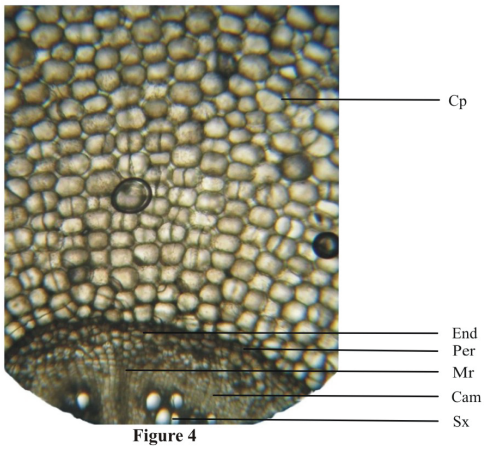


Figure 4

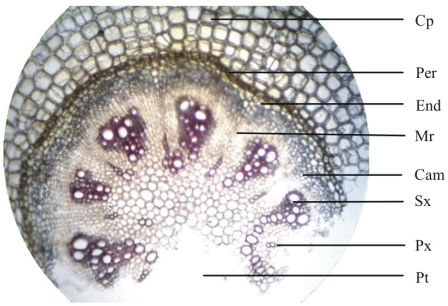


Figure 5

Figure 3, 4, 5 and 6: T. S. of root showing single enlarged portions

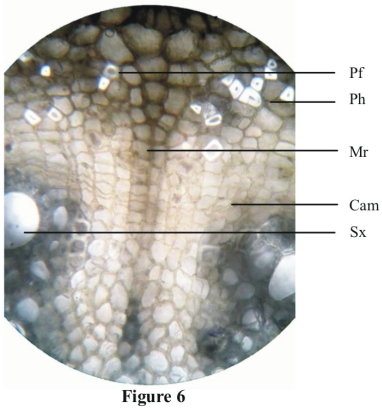
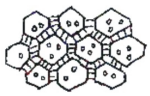
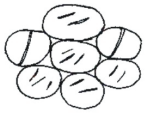


Figure 6

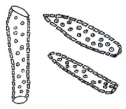


Compactly arranged thick walled pitted hexagonal cells of hypodermis

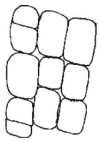
Fragments of thick walled acicular fibres and entire isolated thin walled fibres pointed at the both end



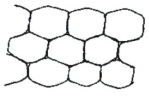
The fragments of cortical parenchyma embedded with acicular crystals of calcium oxalate



Fragmets of border pitted vessels and trachieds



Groups of oval to rectangular longitudinally cut cortical parenchymal cells exhibiting transverse partition at places



Darkbrown coloured hexagonal cells of epiblema

Figure 7: Powder characters of Nelsonia canescens root

Abbreviations: Cam-Cambium, Cp-Cortical parenchyma, End-Endodermis, Ep-Epiblema, Ex-Exodermis, Mr-Medullary rays, Per-Pericycle, Pf-Phloem fibre, Ph-Phloem, Pt-Pith, Px-Primary Xylem. Rh-Root hairs, Sx-Secondary Xylem,

hill ranges, Bolangir, Orissa, India, in fully matured condition and the material being identified by local herbal experts and was being authenticated by the experts of our institute (I.P.G.T. & R.A.). The correct identity and authenticity of the plant *Nelsonia canescens*.(Lam) Spreng. (Acanthaceae) was done by studying its morphological characters and comparing them with the characters mentioned in various floras[4,10,2,11]. Roots were washed properly under running water to make it free from foreign matter like sand, soil etc. Matured root were dried in a shade, powdered to 60# and few were preserved in solution of FAA (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5)[12]. Herbarium was also prepared and submitted to Pharmacognosy museum of I.P.G.T. & R.A., Jamnagar vide Herbarium no. 6002 for future reference.

Pharmacognostic studies

Morphological characters were studied by observing them as such and also by using dissecting microscope. For diagrammatic Transverse section (T.S.) the entire section of the root was taken and cleared with chloral hydrate. For detailed microscopical observation, Free hand, thin transverse sections were taken and were treated as mentioned above for the diagrammatic sections. For lignified elements, the sections were stained with Phloroglucinol and Hydrochloric acid.

The microscopic characters of various sections were drawn with help of Camera Lucida. Photomicrography of transverse sections of root was performed using canon ixus 130 camera. The sections were stained with various reagents like Iodine for starch grain, Phloroglucinol + HCL for lignified contents etc [13].

RESULTS AND DISCUSSION

Macroscopical characters

Cluster of cylindrical long roots measuring 12-15 cm long and 0.8-1.2 cm in diameter. Surface is longitudinally wrinkled. At places rootlets and root scars are present. (Figure 1) Root fracture is rough and short. Roots are creamish brown in colour. They are having sweet taste and characteristic odour.

Microscopical characters[Figure 2-6]

Diagrammatic T.S. of the root is circular in outline and shows wide parenchymatous cortex, distinct endodermis, pericycle embedded with groups of fibres. Wide pith encircled by stellar tissue. Detailed T.S. shows outermost layer of epiblema with broken or unicellular long root hairs. Underneath these lie 5-6 rows of compactly arranged suberized cells of hexagonal thick walled exodermis followed by wide parenchymatous cortical zone consisting of cells exhibiting partition at places; spherical to oval thick walled cells embedded with fine and small acicular crystals and few prismatic crystals. Underneath this endodermis lies a layer of pericycle. The cells are bigger in size, varying sizes and shapes and embedded with microsphenoidal crystals of calcium oxalate, 1-3 in rows, phloem consist of phloem parenchyma, at places prismatic crystals of calcium oxalate embedded, isolated or groups of 2-3 phloem fibres. At places they run radially also, thick wall with narrow lumen with striated wall. It consists of phloem fibres and parenchyma. Cambium is well developed, distinct running continuously. Xylem shows alternate rows of groups of secondary xylem showing exarch condition. Secondary xylem consists of isolated radially arranged xylem vessels of different sizes and shapes of xylem parenchyma. Medullary rays arising from the primary bundles, uni to tri seriate is getting wider towards periphery. Pith is very wide and parenchymatous just like cortical cells, at places the inner wall of the cell get detached and embedded in different form in cell cavities.

Powder characteristics[Figure 7]

Dark brown coloured hexagonal shape cells of epiblema attached at places with unicellular thin walled long root hairs, the cells lying under this in surface view shows compactly arranged thick walled pitted hexagonal cells of hypodermis, fragments of cortical parenchyma embedded with acicular crystals of calcium oxalate, fragments of thick walled acicular fibres and entire isolated thin walled fibres pointed at both the end, fragments of border pitted vessel & trachieds, groups of oval to rectangular longitudinally cut cortical parenchymal cells exhibiting transverse partition at places and acicular crystals of calcium oxalate scattered as such throughout.

CONCLUSION

In the parenchymatous cortical zone, inner wall of the cell get detached and embedded in different form in cell cavities; spherical to oval thick walled cells embedded with fine and small acicular crystals. Xylem shows alternate rows of groups of secondary xylem showing exarch condition. Medullary rays arising from the primary bundles, uni to tri seriate is getting wider towards periphery

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Structural Peculiarities of the Vegetative Organs of the Species of Vinca

(*V. minor* L and *V. Herbacea* waldst et Kit)

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Plant Vinca grows in broad-leaved forests, shrubs and on dry slopes. At some place it produces bigger bush woods. It is native to the middle and south Europe. It is decorative and medicinal plant. Generally the family of Vinca – Vinca L unites 7 species. There are two species commonly spread in Georgia: V.herbacea Waldst et Kit and V.pubescens. The plants including in the genus of Vinca – Vinca L. are characterized by perennial, salverform, simple and broad (20-30 mm long) flowers and free stamen.

The aim of the reasearch was to investigate the anatomy of morphological elements of *V.minor* L. and *V.herbacea* Waldst et Kit and accordingly to outline the microstructural features of mentioned species in order to exclude the mistakes and inaccuracy while fixing the identity of the raw material[1-8].

The samples were obtained in the conditions of middle moisture in Tbilisi in 2011y. Cross and longitudinal sections are made by upper ground and under ground organs of the middle part of the plant. Preparatory strips were made in live unfixed material by hand – using a sharp razor. The samples were painted in light safranin solution for 24 hours and were placed on a glass in the drop of glycerin. For micro-structural researches there were used light (Carl Zeiss, Jeneval) and stereoscope (MBC-2) microscopes. The photo-material is fixed by digital photo camera (Canon Digital IXVS75). Selected photos are processed by computer program"Adobe Photoshop 2007".

Leaf: The leaf mesophyll of both species of Vinca belongs to dorzoventral type. It is characterized by thin cuticle, single-layered upper and lower epidermis and middle sized cells. The tissue of upper epidermis of *V. herbacea* exceeds the size of *V. minor*. *V.minor* has double-layered palisade Parenchyma and *V. herbaea* has single-layered but elongated shape. Both species are characterized by palisade Parenchyma that stimulates the activity of chlorophyll. Cloud-shape Parenchyma is characterized by rounded, thin membrane structure. The species of *V.herbacea* is characterized by abundance of conductive bundles. Conductive bundles are concentric type, sometimes there is anatomical arrangement. The basic cells of upper and lower epidermises of the leaf of the species of Vinca for research belong to different type of curvilinear clan. The apparatus of ventilation system – stoma is placed only in the tissue of lower epidermis. The basic cells of upper epidermis of V. minor belong to the 2-order of curviwalled and those of *V.herbacea* belong to the first order of curviwalled order. The basic tissue of their lower epidermis belongs to curvilinear clan. There are characterized the basic cells of the 3-order of curviwalled for *V.minor* and the based cells of 2-order of curvilinear for *V.herbacea*. For both species there are characterized difficult, disectional (paracytal) stomas. According to satellite shapes they belong to pararugocyte type.

Major vein of the leaf of the mentioned species is bow-shaped, a single-layered thin epidermis cuticle. The cells of lower epidermis of *V.herbacea* have papillary shape; trichomes are placed in units on upper hoop of only *V.minor*, they belong to simple, single-celled coned type. For major vein of the leaf of both species of Vinca is characterized intermixed collenchymas tissue that is placed on in the peripheries of the major tissue. The basic parenchyma of the major veins is active and is characterized by the cells of dense structure, thin membrane, and rounded, cut-shaped cells. The structure of basic tissue of *V.herbacea* is distinguished in upper periphery, there are fixed the habitus and disposition characteristic for the tissue of parenchyma cells. The conductive pile of the major vein of both species is bow-shaped, difficult, open bicolateral type. Phloem fibbers are placed individually and in groups as well only in the side of lower arch. In the tissue of wood there is differentiated rarely circle-shape, and often vessels with thick membrane. For the major vein of both species there are characterized the radial rays of a single-layered type of wood (Picture 1 & 2).

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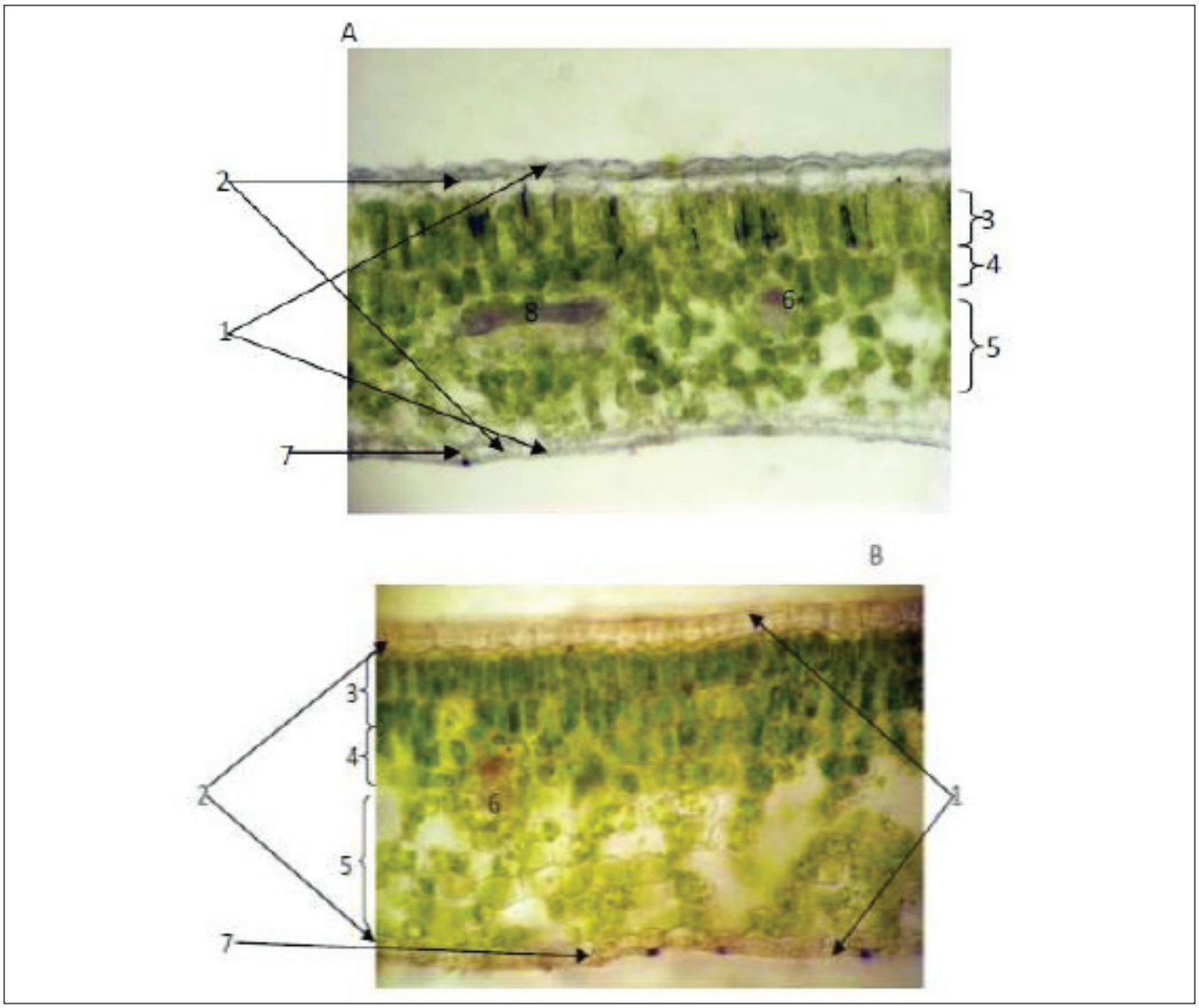
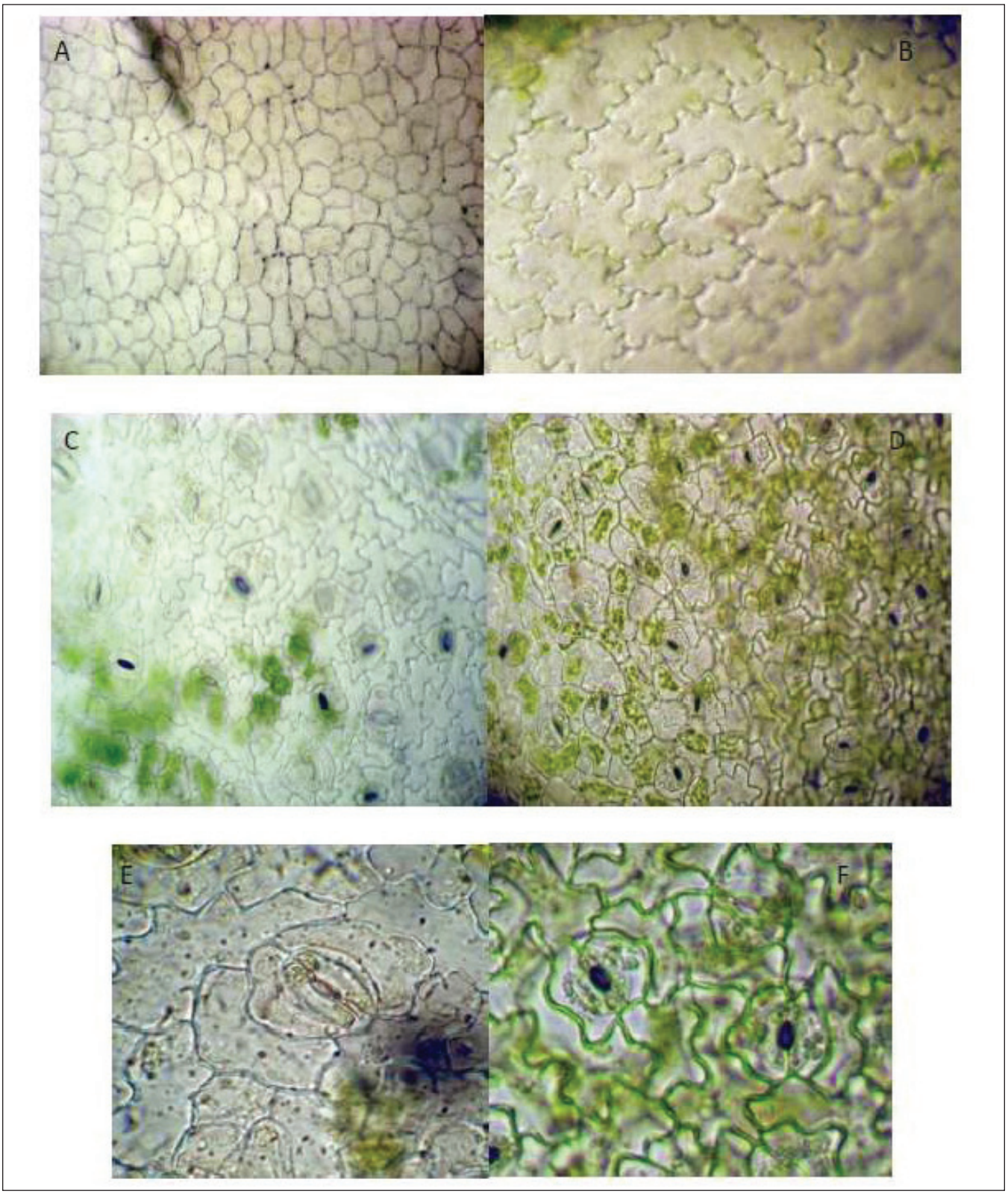


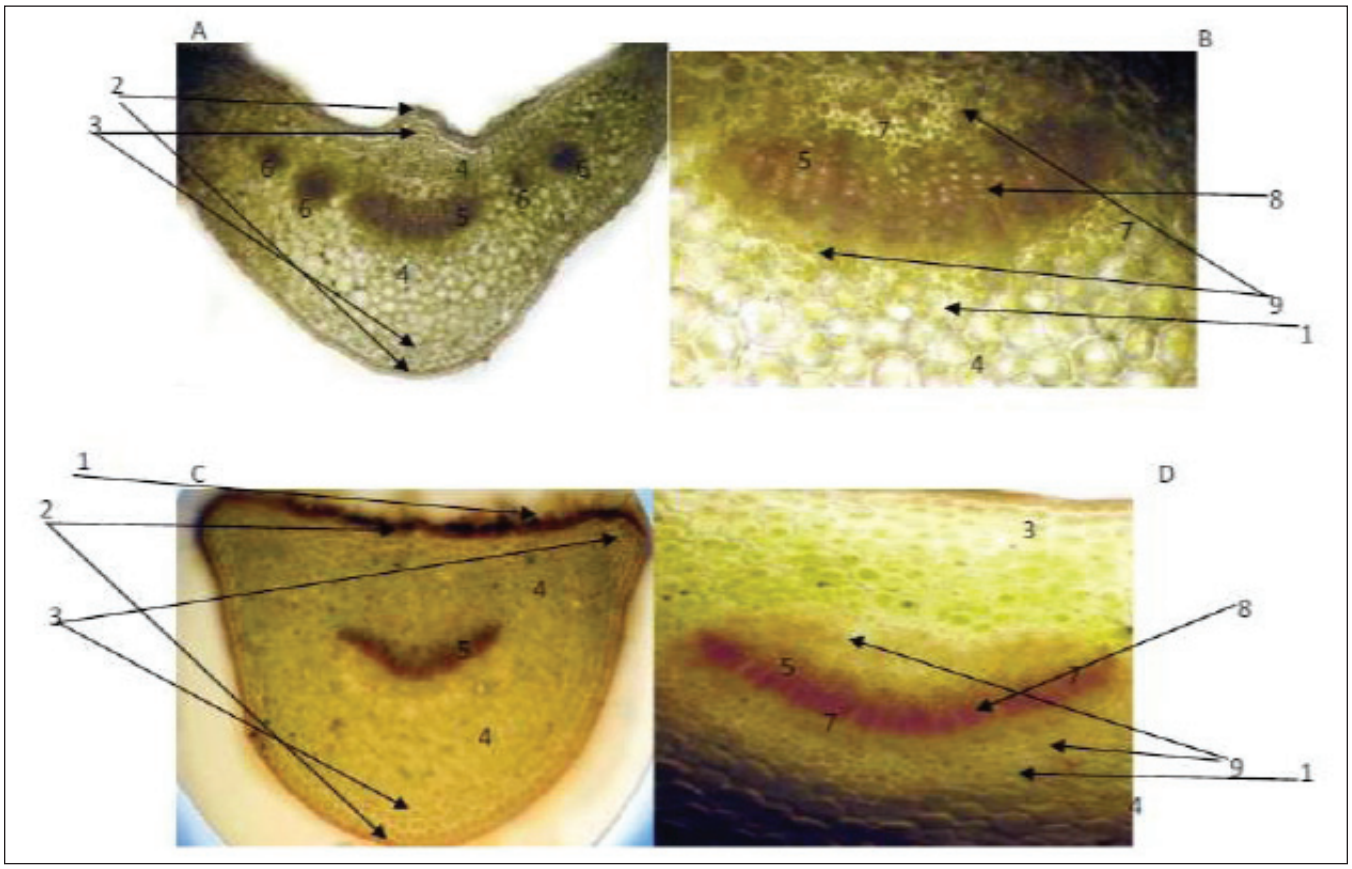
Picture 1: A) *V.herbacea*- B) *V.minor*-Cross section of leaves
1. Cuticule 2. Upper and lower epidermis; 3. Parenchime; 4. Palisade Parenchyma; 5. nebolous parenchyma; 6. conductive bundle; 7. stoma; 8. anastomosis;

PETIOLE

The petiole of mezophecyole zone of the leaf of research species of Vinca is bow-shaped; *V.herbacea* is characterized by elongated lobe and cone-shape rib; *V.minor* has comparatively small arch on the rib and lobes. Both species are characterized by thin united cuticle and a single-layered epidermis. The rib and lobes of *V.minor* petiole is actively covered by single-celled, cone-shape trichoma. On the covering tissue of petiole of the species to research there is the apparatus of ventilation system – stoma. In petioles of both species the intermixed collenchyma is active and especially in lobes and sinuses. The main tissue of the petiole of mentioned species is presented by rounded shape cells of dense structure and thin sheath. In the parenchyma of major tissue there are idioblast cells, complex grains of chlorophyll and starch, raphides. Conductive bundles of both species of Vinca are bow-shaped, conductive bundle of *V.minor* is placed in the centre of petiole and *V.herbacea* is characterized by central and lateral additional conductive bundles. Conductive bundles of both species are complex and belong to open bicolateral type. Phloem fibbers in both sides make bundles, there are also fixed idioblast cells. Wood is presented by libreform, spiral, rounded-spiral and rarely stair like bundles. Radial rays of wood are single or double-layered. Additional bundles of *V.herbacea* belong to concentric type. Besides, there are also differentiate transitive forms of conductive bundles between concentric and bicolateral ones (Picture 3).



Picture 2: F Upper and lower epidermis of leaves of *V.herbacea* and *V.minor*
A) V. herbacea; B) V.minor: Upper epidemis
C) V.herbacea; D) V.minor: lower epidermis
E) V.herbacea; F) V.minor: stoma of disectic type



Picture 3: cross dissection of petiole (mezopetiole) *V.herbacea* and *V.minor*
A.B.) V.herbacea; C.D.) V.minor: 1. Simple trichoma; 2. epiderma; 3.Collenchyme; 4.Main parenchyme; 5. conductive bundle; 6.additional bundle; 7. Phloem; 8. rounded-spiral and rarely stair like bundles; 9. Floem fibbers; 10. idioblast cells

STEM

The stem of *V.minor* of Vinca species has round shape and it is characterized by two weakly expressed opposite ribs, as for *V.herbacea* it is characterized by cone-shape bent particles at the ends of opposite ribs of oval form. Nether species has any kind of covering; cuticle is thin and united. The epidermis cells are single-layered and dense structure. There are stomas in epidermis tissue of both species. Collenchyma of plate type is basically of double-layered, but in cone-shaped sinuses there is only placed the collenchyma tissue. Parenchyma of stem of both species is characterized by middle sized thin cells of dense structure. They have more active central cylinder tissue than membrane parenchyma. The tissues of phloem and wood are the same. There are fibber bundles of phloem presented actively in phloem and there are iodblast cells among them. The phloem fibber is bounded by double-layered cambium. There is differentiated the parenchyma of thick sheath in the wood, rarely circular and stair like and also spiral vascular bundles. Radial rays of wood are single or double layered. The parenchyma of wood is bounded by perimedular tissue and the bundles of inner phloem. The basic tissue of the central cylinder of both species is identical – slightly dense, rounded, with thick sheath and middle sized cells. In the parenchyma of central cylinder there are fixed as iodblast cells as raphides and other kind of cell bodies (Picture 4).

RHIZOME

Underground stem of the species of Vinca for research – rhizome is characterized by thin and weak cuticle, single-layered epidermis placed like palisade cells and having elongated square shape. Plate-like collenchyma is double or triple layered and united. In the rhizome of *V.herbacea* the wood parenchyma and conductive system are separated by endoderm tissue that is characterized for underground stem of *V.minor*, at the same time this species is characterized by differentiated and active conductive system, it may be said that phloem and phloem fibber are as active as wood elements; there are distinguished parenchyma and vascular bundles of wood of primary and secondary development; in primary wood vascular bundles have thick sheath and in secondary wood they

are circle-spiral and spiral. Inner phloem, phloem fibbers and perimedial tissue are more active than in rhizome of *V.herbacea*. In underground stem of *V.herbacea* due to endoderm tissue the conductive system is concentrated. The tissue of inner phloem isn't fixed and accordingly neither - phloem fibbers. Parenchyma of central cylinder of both species is characterized by rounded cells with thin sheath (Picture 5).

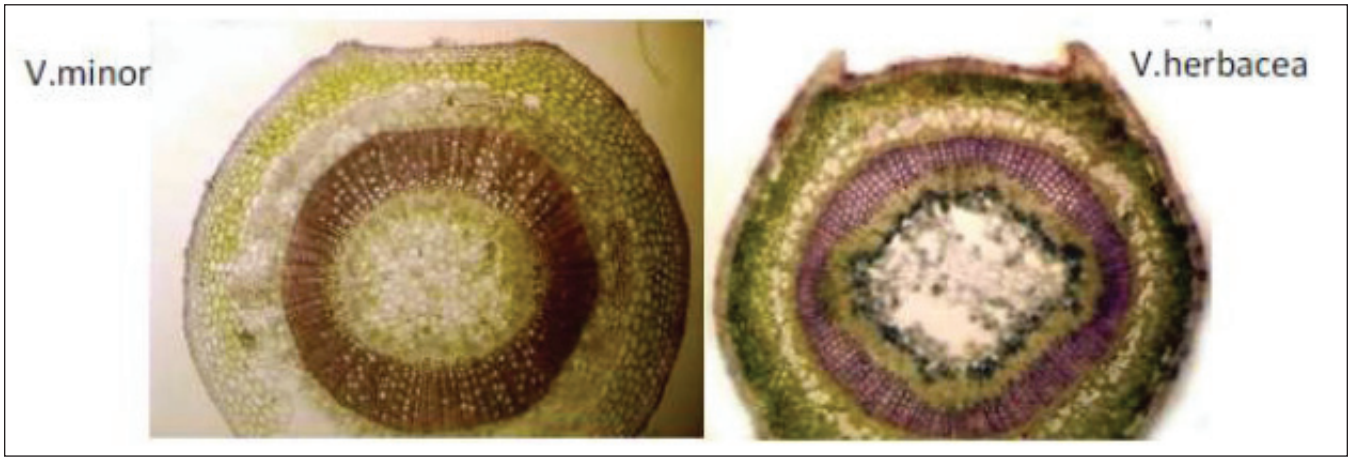
ROOT

In the primary root of Vinca to research there is differentiated single layered epidermis, primary wood parenchyma, endoderm and conductive bundle of concentric type. Endoderm is single-layered and there are Caspar spots fixed on the cell membrane. Phloem is united and it covers the primary wood tissue. There are pericyclic elements placed in the wood of primary root of the mentioned species of Vinca; Wood parenchyma is presented by libreform, vascular bundles have thick sheath.

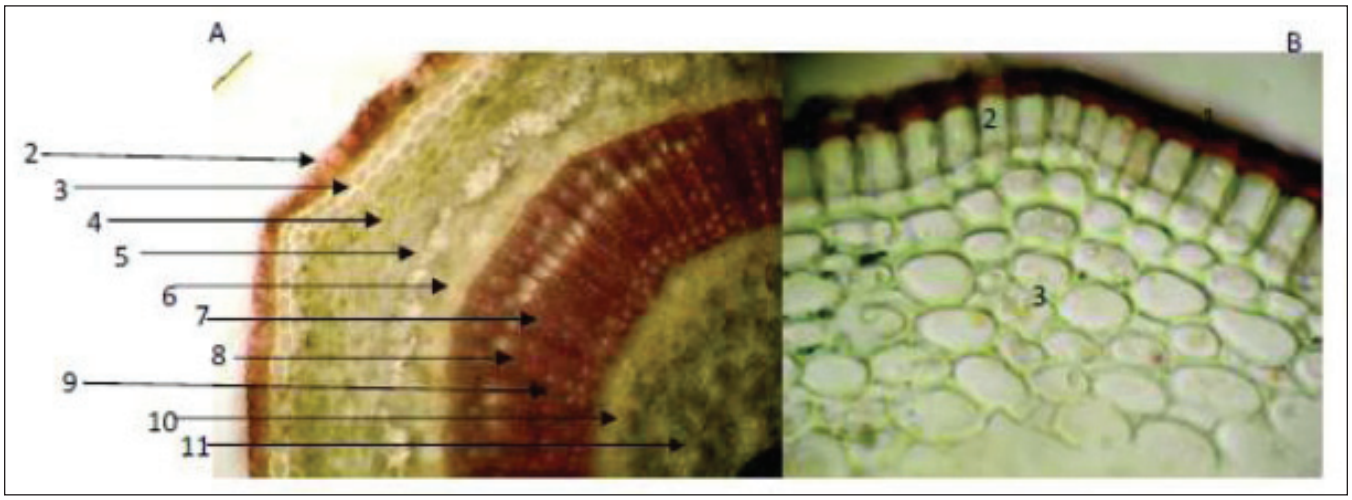
The structure of secondary root of the same species of Vinca is differentiated and accordingly covering, basic and conductive system is very active. There are distinguished the wood elements as well. The wood elements are also distinguished; wood parenchyma and vascular bundles of primary and secondary development. In the wood parenchyma there are actively placed the cell bodies (Picture 6 & 7).

CONCLUSION

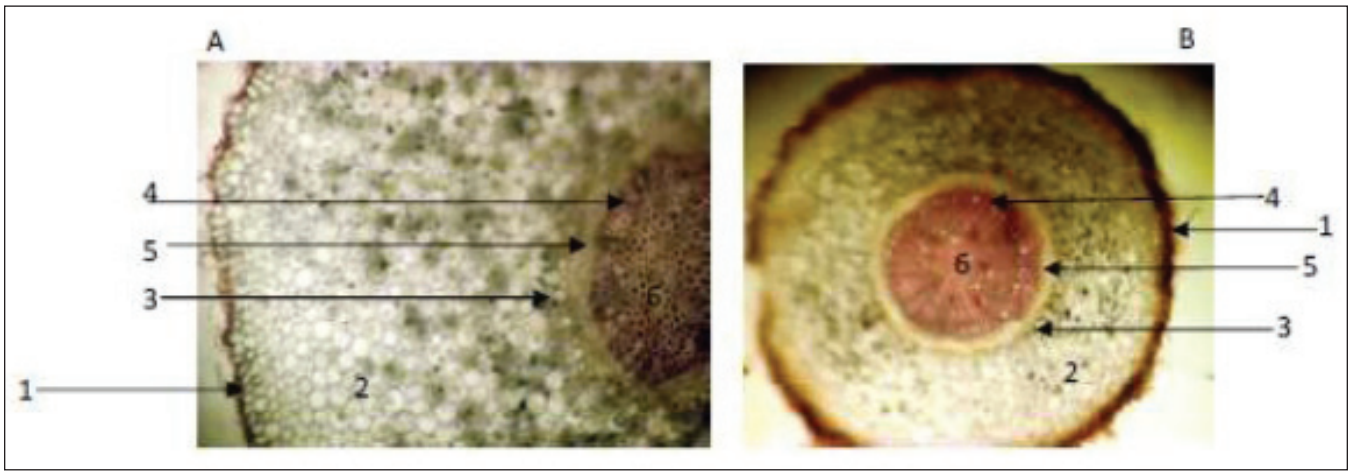
Due to the goal of the research there were expressed the microstructural features of morphological elements of the species of *Vinca V.minor L* and *V.herbacea* Waldst et Kit. The mesophyll of both species is dorsiventral; The basic



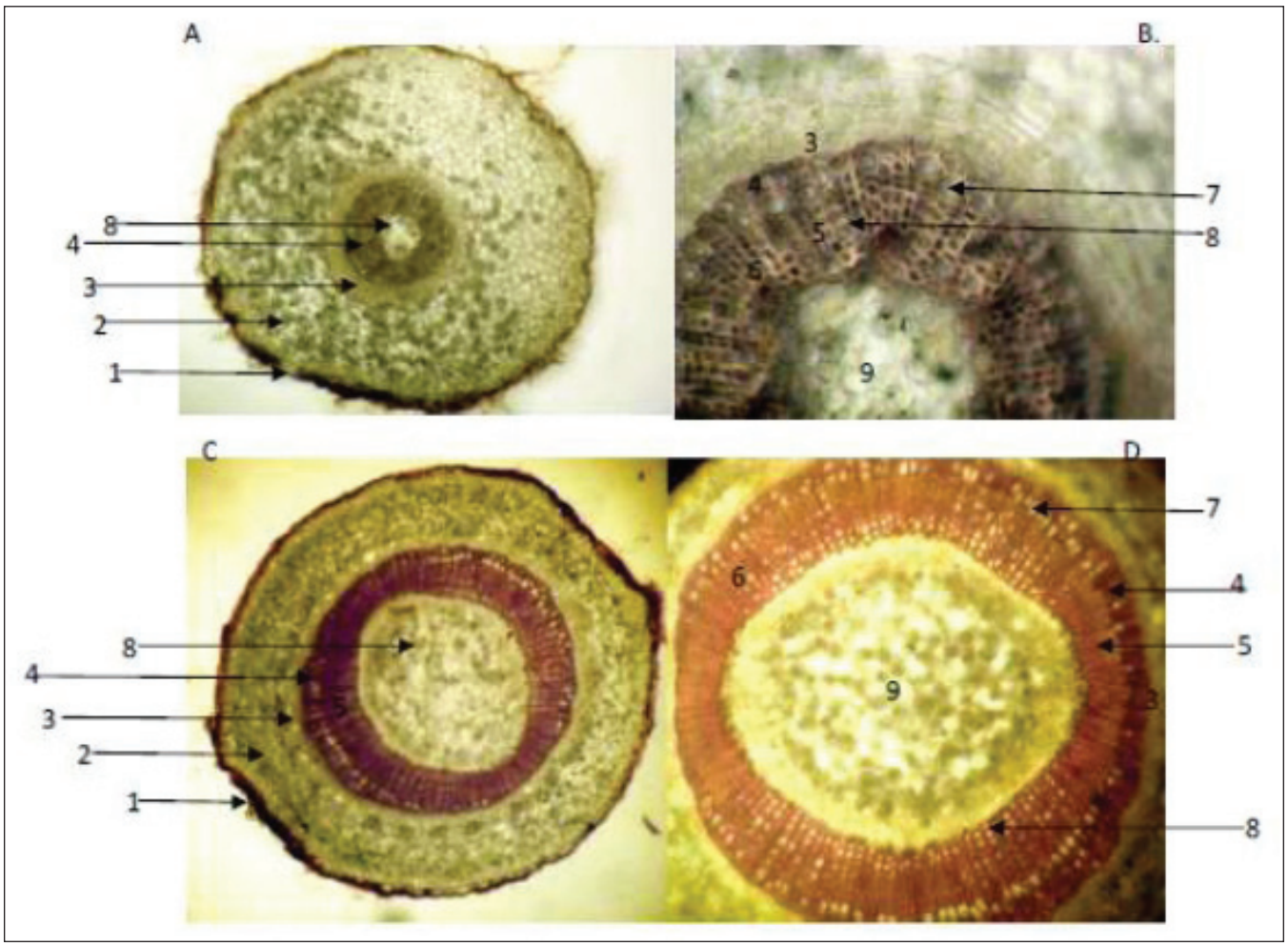
Picture 4: Cross section of stem



Picture 5: Cross fragments of rhizome.
1. Cuticule; 2. epiderma; 3. Colenchyma; 4. parenchyma of bark 5. Fibbers of Phloem 6.Phloem 7.wood 8.radial ray of wood; 9. vessels; 10. Erimedular zone; 11. Parenchyme



Picture 6: Cross dissection of primary root of *V.herbacea* and *V.minor*
A) V.herbacea; B) V.minor: 1. Epiderma; 2. parenchyma of primary bark; 3. endoderm; 4. Peryccyl; 5. Phloem 6. Wood



Picture 7: Cross dissection of secondary root of *V.herbacea* and *V.minor*

cells of upper and lower epidermis belong to curvilinear clan and according to the order –different types of curvi walled – upper epidermis of *V.minor* L - to the 2nd order of curviwalled, and lower – to the 3rd order. The basic cells of upper epidermis of *V.herbacea* belong to the first order of curviwalled and lower epidermis to the 2nd curviwalled order. The ventilation apparatus is placed in lower epidermis in both species and bothare identical, - by new scheme

dysectal (paracytal) and satellite cells are pararegocytal type. The activity of mid rib of both species is defined by collenchyme, rich structure of cell and dence bodies of major parenchyma and strong conductive system. The conductive bundle of mid rib of a leaf of both species is complex and open bicolateral type.

The research of a structure of petiole of research patterns showed that in petiole of *V.herbacea* there is fixed the great influence of inner structure of leaf mesophyll that is proved by existence of palisade cells. This fact is essential as the mentioned species are characterized by small petioles, but the existence of palisade tissue in any organ of the plant is determinant of its activity and inner hardness. So this fact gives positive characterization to the species of *V.herbacea*. At the same time this species is characterized by central and additional conductive bundles along with basic conductive bundles that points on its archaic structure. Basic conductive bundles of both species belong to complex, open bicolateral type. In both cases there are iodblast cells next to conductive bundles.

Nearly identical inner structure is characterized for the stems of the species for research, covering tissue is single-layered and there are small number if stomas there; plate form collenchymas is united; wood parenchyma is of dense structure; phloem fibbers and inner and outer phloem are active; wood tissue where there are placed vascular bundles of different type points on functioning of strong and active conductive-supplier system. The stem plasticity is stipulated by plastic cells (cell bodies) placed in parenchyma of crust and central cylinder. Conductive bundle of complex and bicolateral type is characterized for both species.

According to living conditions, the rhizome of research object (underground stem) unlike stem has developed palisade type epidermis cells. In rhizome the mechanical tissue is more weakly presented and there are no stomas in covering tissue. There was no fixed endoderm.

The root system of mentioned species is characterized by typically similar primary and secondary differentiation, the difference is only in features of structural elements. In primary root the parenchyma of primary crust and endoderm tissue are active where there are Caspar fats; the primary conductive system is concentric type, in case of *V.herbacea* there are fixed procambium cells. The central cylinder of primary cell is wholly filled by wood tissue. In case of secondary root the conductive system is more active and the tissue of phloem and wood are more differentiated. There are expressed the elements of primary and secondary wood; lybreform, circle and spiral vascular bundles. The central cylinder is filled by parenchyma tissue, where there are fixed plastic cells.

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