CHAPTER I

PHARMACOGNOSY

1.1 INTRODUCTION

The use of plants as medicine is as old as human civilization. Men of all ages in both developing and undeveloped countries use plants in an attempt to cure various diseases and to get relief from physical sufferings. India is known as the “Emporium of Medicinal plants” due to the prevalence of several thousands of medicinal plants in different bioclimatic zone (Yoganarasimhan, 2000). The peninsular India probably has the richest and varied flora than other tracks of equal area in India, possible in the world (Gamble, 1967), a phenomenon contributed by its combined effects of its geographical situation and topography. The Western Ghats have been designated as one of the hot spots of global biodiversity. A rich depository of flora with high endemism is found in the Western Ghats. The immense taxonomic diversity of the country throws a challenge to the Indian chemists and biologist to transform the enormous bioresource into economic wealth and intellectual property. The state Tamil Nadu is endowed with a very rich flora due to the various physiographic features and physiognomic factors and different types of vegetation exist in the state. A total of 5640 species of the flowering plants (including 6 gymnosperms) are reported either naturally occurring or cultivated in the state (Nair and Henry, 1983). Among them numerous plants have been reported to be medicinal plants. However, our knowledge of medicinal plants has mostly been inherited traditionally. Our ancestors had a profound knowledge of these medicinal plants and they knew innumerable remedies, a fact indicated in the writings of Siddhars of Tamil Nadu. Had their expertise documented properly it would help the modern man to find more effective prophylactic use of these herbs.
Modernity has brought us several new things and we have discarded certain old beliefs and practices little realizing the adverse impact on our physical well being. Now the side effects of most effective life saving allopathic drugs have recently opened the eyes to look behind for effective non-toxic safe drugs. The modern pharmacologists now strive to learn more about ancient systems of medicine and the vast treasure of the herbal wealth of our country (Pandey et al., 1995). Even today, years after the introduction of modern medicine in our country, the Indian systems of traditional medicine continue to provide medical relief to nearly 80% of our total poor people (Saxena and Tripathi, 1989). It has also been estimated that as many as 90% of the world’s rural population rely on herbal drugs for their primary health care (Yoganarasimhan, 2000).

Thus, research in plants, scientific evaluation and standardization of the new and little known herbals have gained their drive since the upsurge of interest in the molecular aspects of the drugs.

Meliaceae or the Mahogany family is a flowering plant family of mostly trees and shrubs (and a few herbaceous plants, mangroves) in the order Geraniales. Meliaceae is a woody family widely distributed throughout the tropics and subtropics, with only slight penetration into temperate zones; they occur in a variety of habitats from rain forests and mangrove swamps to semi-deserts. It consists of about 50 genera and about 500 species (Pennington and Styles, 1975). About 20 genera and over 75 species of Meliaceae are reported from India. Common Indian species include Azadirachta indica (Neem), Melia azedarach and Toona ciliata. The family contains many important medicinal, timbers and ornamental trees. Other uses of Meliaceae comprise shade and street trees, fruit trees and sources of biologically active compounds (Mabberley et al., 1995). Among other secondary metabolites, Meliaceae
synthesize and accumulate bitter and biologically active nortriterpenoids, which are also known as limonoids and meliacins. These and other compounds have aroused considerable commercial interest due to their insect-antifeedant (Simmonds et al., 2001), insect-repellent (Shukla, et al., 1997), insecticidal (Greger et al., 2001), molluscicidal (Singh et al., 1998), antifungal (Engelmeier et al., 2000), bactericidal (Aboutabl et al., 2000), and anti viral (Singh et al., 1988) activities as well as their numerous medicinal effects in humans and animals (Benencia et al., 2000).

The genus *Naregamia* of Meliaceae is represented by only one species in India (Hooker, 1872), *Naregamia alata* Wight & Arn. It is commonly called as Goanese Ipecacuanha. It is a rare under shrub, 15-45 cm tall, found mainly on rocky or grassy slopes in western peninsular India upto 1000m height. It has been used in traditional medicines in India and elsewhere in the treatment of rheumatism, itch, malarial and chronic fevers, wounds, anaemia, enlarged spleen, ulcers, vitiated conditions of pitta and vata, halitosis, cough, asthma, splenomegaly, scabies, pruritis, dysentery, dyspepsia and catarrh. In Southern India, a small dose of the plant is used as an expectorant. The plant is acrid, sweet, cooling, aromatic alexeteric, emetic, expectorant and depurative (Biju, 2014). The root of *Naregamia alata* is used to cure asthma, bronchitis, biliousness and ulcers. *Naregamia alata* has been reported to be a constituent of an Ayurvedic drug ‘Pittapapda’ (Rajopadhye and Upadhye, 2013).

The genus *Walsura* Roxb., belonging to the family Meliaceae comprises 10 species in India (Hooker, 1872) but Willis (1955) reports the occurrence of 15 Indo-malayan species of the genus. *Walsura trifoliata* (A. Juss.) Harms. (Syn: *Walsura piscidia* Roxb., *Heynea trifoliata* A.Juss.) is an evergreen tree distributed widely in the tropical areas of Asia, such as Southern China, India, Malaysia, and Indonesia (Chetty et al., 2008). It grows on dry deciduous forests of 200 to 300m height. This
The plant is well reputed in traditional system of medicine and used by tribal people to treat various diseases like skin allergies, astringent and diarrhoea (Pullaiah and Rani, 1999). The bark of the plant is reported to possess stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison (Anonymous, 1976).

Pharmacognosy literally means knowledge of drugs or pharmaceuticals, which deals with the drugs of vegetable, animal and mineral origin. It may be defined as an applied science that deals with “biological, biochemical and economical features of natural drugs and their constituents”. Pharmacognosy helps to study the identification of the source of the material forming drug, description of its morphology and anatomy, investigation of its potency, purity and freedom from admixture, devising the methods of cultivation, prescribing the details of collection and preparation processes and studying the constituents of the drug and investigation of their physico-chemical properties (Wallis, 1985).

The pharmacognostical studies of the herbal drugs have become imperative for several reasons. As per the WHO norms, every drug has to undergo botanical standardization, particularly macroscopic and microscopic characterization which constitutes the major part of pharmacognosy. This primary step enables the researcher in phytodrugs to affirm. The botanical standardization is based upon the tenet that certain microscopic characters are specific and restricted in distribution (Metcalfe and Chalk, 1979). Microscopic parameters, though limited in their application under certain circumstances, have still highly reliable diagnostic values and play appreciable role in the herbal drugs.

A perusal of literature on the much-valued medicinal plants Naregamia alata and Walsura trifoliata of Meliaceae reveals a lacuna in the pharmacognosy and other
parameters. This fact induced the present investigation of the micro morphological standardization of the folklore drug to put forth a protocol of anatomical features.

1.1.1. AIM AND SCOPE OF STUDY

Microscopical analysis of plant is a well established and useful criterion for the identification and authentication of medicinal plants (Jackson and Snowdon, 1990). It has the advantage of requiring only small quantities of material, economical and can provide a rapid tentative identification, prior to confirmation by analytical techniques. Microscopic specimens prepared can be compared to microscopic descriptions present in the monographs, or in a visual atlas of plant material.

Most of the species of Meliaceae have medicinal properties. Currently however, no such description or standardization is available for *Naregamia alata* and *Walsura trifoliata*. A microscopic illustration describing diagnostic characteristics on such plants will be helpful fulfilling a need for information on plant identification and quality control.

In the present study, the time renowned microtechnical procedures were employed and data pertaining to morphological and anatomical characteristics of the selected taxa were retrieved. Every essential observation was supplemented by supporting photographs. Customary parameters of pharmacognosy such as powder drug analysis and powder microscopy were given due importance. These studies will offer the scope for easy and accurate identification of the specimen either in incomplete or fragmentary form.

1.1.2. PLANT PROFILE:

The following plant species were selected for the present investigation.

The plant names, localities and dates of collection are furnished below:
1.1.2.1. *Naregamia alata* Wight & Arn.

**Vernacular Names**

**Tamil** : Nilanarai

**Malayalam** : Nilanarakam

**English** : Goanese Ipecac, Goanese ipecacuanh

**Locality** : Petchiparai, Kanyakumari District, TamilNadu.

**Date of Collection** : September, 2010

**Botanical descriptions:**

**Habit** : Rare shrub (Fig. 1.1), 15-45 cm tall

**Leaves** : Leaves are divided into three leaflets (Fig.1.2), each of which is wedge-shaped-ovate, quite entire, and stalkless. Petiole 1-3cm long winged; leaflets oblong-ovate, base acute, inequilateral, apex acute; nerves 5-6 pairs, petiolule inconspicuous.

**Inflorescence /flower** : White flowers (Fig. 1.4), 2.5-5 cm long, arise solitary in leaf axils. Sepals five, 5 mm long, calyx persistent. Petals are 5, very long, strap-shaped, distinct, and free from the stamen tube. Filaments are united into a long tube, inflated and spherical at the tip (Fig. 1.3).

**Fruit & seed** : Capsule is 3-cornered, 3-valved, valves circular, 8 mm long. Seeds 2 in each cell, curved. Flowering: April-May.

**Ecology** : Found mainly on rocky or grassy slopes in western peninsular India.
1.1.2.2. *Walsura trifoliata* (A. Juss.) Harms.:  

**Synonym:**  
- *Heynea trifolia* A. Juss.  
- *Walsura piscida* Roxb.  
- *Walsura tabulata* Hiern.

**Vernacular names:**  
- **Tamil:** Walsura, Cheddavokko, Kanjimaram  
- **Malayalam:** Perillapacha; Perilla ppichu  
- **Kannada:** Male sagade  
- **Locality:** Karaiyar - Papanasam, Tirunelveli.  
- **Date of Collection:** October, 2010

**Botanical descriptions:**  
- **Habit:** Trees up to 15 m tall (Fig. 8.1).  
- **Trunk & Bark:** Bark pale brown, shallowly fissured, lenticels rusty brown; blaze pink (Fig. 8.6).  
- **Branches and branchlets:** Branchlets slender, terete, lenticellate, glabrous.  
- **Leaves:** Leaves compound, trifoliate (Fig. 8.2), alternate, spiral; rachis up to 5 cm long, triangular, pulvinate, glabrous; petiolule of side leaflets 0.4-1 cm long and middle leaflet with 1.3-3 cm long, swollen at both ends, angled or subterete; lamina 4-15 x 2.5-5 cm, variable in shape, narrow oblong to elliptic or narrow obovate, apex acuminate with retuse tip or rounded with retuse, base acute to cuneate, margin entire, chartaceous to subcoriaceous, glaucous beneath; glabrous; midrib flat
above; secondary nerves 7-13, gradually curved; tertiary nerves broadly reticulate, slender.

<table>
<thead>
<tr>
<th>Inflorescence /</th>
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<tbody>
<tr>
<td><strong>Flower</strong></td>
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<tr>
<td>Inflorescence terminal or axillary panicles (Fig. 8.5); flowers greenish-yellow. Flowering: January-March.</td>
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<tr>
<td><strong>Fruit and Seed</strong></td>
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<tr>
<td>Berry ovoid (Fig. 8.4), to 1.3 cm long; seeds 1-2, pale brown, enclosed in a white fleshy aril.</td>
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<tr>
<td><strong>Ecology</strong></td>
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<tr>
<td>Understorey trees in wet evergreen to semi- evergreen forests up to 1200 m.</td>
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<td><strong>Distribution</strong></td>
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<td>India and Sri Lanka; in the Western Ghats – South and Central Sahyadris.</td>
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1.2. REVIEW OF LITERATURE

Pharmacognostic studies on Indian medicinal plants have gained momentum in recent years following an awareness of the values of indigenous and traditional systems of medicine. Since the members of Meliaceae possess valuable medicinal properties, pharmaceutical chemists and medicinal botanists have started paying attention to the members of this family.

The presence of unicellular, multicellular and glandular trichomes with varying shapes, single layer of palisade and difference in vascular bundle shape have been observed as common characters of family Meliaceae (Metcalf and Chalk, 1957).

Presence of cylindrical or single crescent shaped vascular bundle, various types and sizes of secretory cells and solitary/clustered crystals have been observed as common characters of petiole of family Meliaceae by Alexandra et al., 2008.

Ahmad et al., 2010 recognized the polygonal cells with smooth walls in leaf epidermis in Melia azedarach.

Jafari et al., 2013 studied the peltate trichomes in the leaf epidermis of Azadirachta indica and Melia azedarach. Fragments of lignified vessels with spiral thickening are found in the leaves of both plants.

Mensah, 2012 reported the occurrence of numerous stomata on lower epidermis in Azadirachta indica. The epidermis is relatively thin. The palisade mesophyll is composed of two layers of elongated closely packed cells. The spongy mesophyll is composed of loosely arranged cells with air spaces.

Lagos et al., 2007 studied the leaf of Trichilia catigua (Meliaceae), and reported that the leaf midrib is convex and traversed by one collateral vascular bundle, encircled by a complete sclerenchymatic sheath. The stem bark is distinguished into
periderm, consisting of suber, phellogen and various layers of phelloderm. The fibres form small bands and contain many calcium oxalate prisms.

Attarde *et al.*, 2010 reported the presence of more amounts of cluster type calcium oxalate crystals in the leaflets of *Soymida febrifuga*.

Ibrahim *et al.*, 2006 examined the powdered bark of *Khaya* species. This examination revealed different types and shapes of lignified sclereids, abundant distribution of prismatic calcium oxalate crystals, druses, lignified fibres, medullary rays and parenchyma cells which may contain starch grains.

Singh *et al.*, 2012 studied the powdered bark material of *Toona ciliata* and showed the presence of pointed lignified fibres, scattered stone cells, few in groups and multicomponent starch grains.

Bhat, 1994 studied the vascular tissue of *Aglaia barberi* and according to him the phloem rays were 1 to 3 seriate more commonly biseriate ranging from 100 to 450µm in height and 10 to 35 µm in width. Phloem rays were closely spaced and heterogenous with usually a single marginal row of square cells. Ray heterogeneity was sometimes less pronounced.

The present proposed study is justified since it may throw immense light on the anatomical and phytochemical dimensions of the two selected species. The study will provide a protocol for the pharmacognostic standardization of the two species.
1.3. MATERIALS AND METHODS

1.3.1. Collection of specimens:

The plant specimens were collected from the southern Western Ghats, and identified and checked by Dr. Chelladurai, Retired Research Officer, Botany, Scientist C cadre, Central council for Research in Ayurveda and Siddha, Government of India. The Flora of presidency of Madras (Gamble, 1997) and Flora of Tirunelveli Hills (Manickam et al., 2008) were referred for the identification of the chosen plants. The taxonomic identity of the medicinal plants were confirmed by comparing the collected voucher specimens with those of known identity and voucher specimens deposited in the Herbarium (SJCH 935 & 936) at PG and Research department of Botany, St. John’s College, Palayamkottai, Tamilnadu.

Preparation of specimens

Healthy plants and normal organs were collected carefully. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of Tertiary Butyl alcohol (TBA) as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was carried out by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue (O’Brien et al., 1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; some cytochemical reactions were
also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies and other necessary sections were also stained with safranin and Fast-green and KI (for Starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell components were studied and measured.

**Photomicrographs**

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features were given as in the standard Plant Anatomy books (Esau, 1964).

**1.3.2. Physicochemical parameters:**

The following physico-chemical parameters were determined by standard methods (Anonymous, 1996).
Ash values

Ash values are indicative to some extent, of care taken in collection and preparation of drug for market and of foreign matter content of natural drug. The purpose of ash preparation is to remove all traces of organic material interfering in an analysis of inorganic elements.

a. Total ash:

Total ash was determined as formulated by the Association of official analytical chemists (Horwitz, 1980). 2 gm of the sample was taken in a silica crucible which had been previously ignited and cooled before weighing. The ignition was repeated until constant weight was obtained.

b. Water soluble ash:

The ash was boiled with 25ml of water and was filtered through an ashless filter paper (Whatman 41). It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble ash was weighed. The water soluble ash was calculated by subtracting the water insoluble ash from the total ash.

c. Acid insoluble ash:

It was determined by boiling the water insoluble ashes with 25ml dilute HCl for five minutes and filtering through an ashless paper (Whatman 41). The filter paper was ignited in the silica crucible, cooled and acid insoluble ash was weighed.

d. Loss on Drying:

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. 5 gm of powdered sample was weighed and placed in a crucible of silica. The crucible was cleaned and dried and weight of empty dried crucible was taken. The powder was
spread as a thin uniform layer. The crucible was placed in the oven at 105°C. The powder was dried for 2 hours and cooled in a desiccator to room temperature and the weight of the cooled crucible plus powder was noted.

1.3.3. Solubility:

Solubility was determined by standard methods (Anonymous, 1958).

a. Alcohol soluble extractive values

5gm of air dried, macerated and coarsely powdered sample was soaked with 100ml of alcohol in a closed flask for twenty four hours, shaking frequently for six hours and was allowed to stand for eighteen hours. It was filtered rapidly, taking precautions against the loss of alcohol. 20ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish. Again it was dried at 105°C to obtain constant weight. The percentage of alcohol soluble extractive was calculated with reference to the air dried sample.

b. Water soluble extractive values

It was proceeded as directed for alcohol soluble extractive, using water instead of alcohol.

1.3.4. Extractive values (Successive extraction):

The freshly collected plant material was cut into small pieces. It was dried and coarsely powdered. The powdered material of 5gm was taken in Soxhlet apparatus and successively extracted with n-hexane, petroleum ether, chloroform, and alcohol till the extracts became colourless. Nearly 80% of the solvent was collected and distilled over boiling water bath. The extracts so obtained were further dried in vacuum desiccators and the yield of the extracts was weighed.
1.3.5. Thin Layer Chromatography:

2gm of the sample was soaked in 20 ml of distilled Ethyl alcohol, kept overnight, boiled for 10 minutes and filtered. The filtrate was concentrated and made upto 5 ml in a graduated test tube. 10 and 12µl of this solution was applied on Merck aluminium plate 60 F254 precoated with silica gel of 0.2mm thickness and the plate was developed in Toluene: Ethyl acetate 1:1. After drying, the plate was visualized under UV 254 and 366 nm and photographs were taken. The plate was dipped in vanillin-sulphuric acid reagent and kept in oven at 105°C till the colour of the spots appeared. The photograph was taken.

1.3.6. Fluorescence analysis:

Fluorescence of the drug was observed in day light and UV light (265nm & 365nm) using various solvent extracts of the drug. The powder was treated with neutral solvents like hexane, petroleum ether, chloroform, ethyl alcohol, ethyl acetate, distilled water, acid like 1N HCl and alkaline solution like 1 N NaOH (Chase and Pratt, 1949).

1.3.7. Qualitative organic analysis:

The 90% alcoholic extract of the aerial part of the plant was subjected to qualitative analysis (Overton, 1963 and Harborne, 1973).

The following tests were carried out.

a. Liebermann-Burchard test: (Steroid)

A few mg of the substance in chloroform was treated with a few drops of acetic acid, acetic anhydride, two drops of con.H2SO4 and heated gently. Blue (or) green colour showed the presence of steroid.
b. Noller’s test: (Triterpenoid)

A few mg of the substance in a dry test tube was treated with a bit of tinfoil, 0.5ml of thionyl chloride and heated gently. Pink colour showed the presence of triterpenoid.

c. Alkaloid:

A few mg of the substance in acetic acid was treated with two drops of Dragendorf’s reagent. Red or orange precipitation indicated the presence of alkaloid. Excess reagent was avoided.

d. Phenol:

A few mg of the substance in alcohol was treated with alcoholic Ferric chloride. Any coloration indicated the presence of phenolic compounds.

e. Sugars/Glucosides:

A few mg of the substance was mixed with equal quantity of Anthrone and treated with two drops of con.H$_2$SO$_4$. It was then heated gently on a waterbath. Dark green colour indicated the presence of sugar/glucosides.

f. Quinone:

A few mg of the substance was treated with con. H$_2$SO$_4$ or aqueous NaOH. Colourisation indicated the presence of quinoid compounds.

g. Coumarin:

A few mg of the substance in alcohol was treated with alcoholic NaOH. Yellow colour indicated the presence of coumarin.

h. Shinoda test: (Flavonoid)

A few mg of the substance in alcohol was treated with magnesium turnings and few drops of con.HCl. Red or Pink colour indicated the presence of flavonoid.
i. Ehrlich’s test: (Furanoid)

A few mg of the substance in alcohol was treated with a pinch of paradimethylamine benzaldehyde and a few drops of con.HCl. Red or pink colour indicated the presence of furanoids.

j. Tannin:

A few mg of the substance in alcohol was treated with a few drops of lead acetate. Precipitation indicated the presence of tannins.

k. Test for saponin

About 2gm of the powdered sample was boiled in 2ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.
1.4. OBSERVATION

Microscopic features

1.4.1. NAREGAMIA ALATA Wight & Arn.

1.4.1.1. Leaf:

The leaf has thick midrib which is projecting on both adaxial and abaxial sides. The lamina is thin and dorsiventral (Fig. 1.5). The midrib has thick, short and cylindrical adaxial hump and fairly wide and thick abaxial part. The epidermal layer of the midrib consists of squarish, thick walled cells with prominent cuticle. The ground tissue is parenchymatous and the cells are angular, thin walled and compact (Fig. 1.6).

The vascular system consists of two vascular strands: one strand is small, circular and collateral with prominent sclerenchyma cap. It is adaxial in position. The abaxial strand is larger, bowl-shaped in outline and collateral. It includes several short, parallel lines of narrow angular thick walled xylem elements and small groups of phloem elements located at the lower end of each xylem strand (Fig. 1.6). A thin layer of fibres occurs all along the abaxial part of the vascular strand.

Lamina:

The lamina is smooth and even on adaxial and abaxial sides (Fig. 2.1). The adaxial epidermal layer consists of thick, rectangular cells with thick and smooth cuticle. The abaxial epidermal layer is comparatively smaller, squarish or cylindrical in shape; the cuticle is thin. The mesophyll tissue is differentiated into a thin adaxial row of short conical compact palisade mesophyll cells and abaxial zone of five or six layers of spherical or lobed, loosely arranged spongy mesophyll cells. The lamina is 120µm thick.
Leaf Margin:

The marginal part of the lamina is slightly dilated and bent down. It consists of small squarish epidermal cells with very prominent cuticle (Fig. 2.2). The size of the epidermal cells becomes gradually reduced in size in the abaxial side of the leaf margin. The differentiation of the mesophyll tissues remains unchanged in the leaf margin. The marginal part is 100µm thick.

Epidermal cells and stomata:

The stomata were studied from the surface view of the paradermal sections (Fig. 2.3 and 2.4). The epidermal cells are small and polyhedral in outline. Their anticlinal walls are thin and straight. The stomata are diffuse in distribution. The stomata are cyclocytic type: each stoma will have six or more subsidiary cells which will radiate in all directions from the guard cells. The stomatal complex will appear star shape in surface (Fig. 2.4). The guard cells are 15×20µm in size.

Venation pattern (Fig. 2.5 and 2.6):

The lamina has distinct intra marginal veins and distinct reticulate venation system. The lateral veins are thick and prominent. The vein lets are thin and less prominent. They form vein islets of various shape and size. Within the islets occur the vein terminations. The terminations are either unbranched or branched once or twice. They are long, slender and wavy (Fig. 2.6).

Petiole:

The leaf has decurrent leaf base and in cross sectional view of the petiole there are two lateral wide wings. The petiole consists of adaxial thick, short adaxial hump and wide and thick abaxial part (Fig. 3.1). The petiole is 900µm thick, the adaxial hump is 250µm wide and the abaxial part of the petiole consists of small, squarish epidermal cells with thick cuticle which consists of minute cuticular spines. The
ground tissue is circular, less compact and some of them contain tannin bodies. The vascular system of the petiole includes two prominent strands of which one is adaxial and semi circular and the other is wide and bowl shaped. The adaxial strand consists of several, short parallel rows of small, angular thick walled xylem elements and a prominent horizontal band of phloem elements on the adaxial side. The strand being protected by a thick arc of lignified fibres. Abaxial strand is wide and thicker it is also collateral with upper xylem and lower arc of phloem elements. The xylem elements are in short, parallel rows comprising narrow, thick walled xylem elements and phloem arc includes small isolated clusters of sieve elements (Fig. 3.2).

1.4.1.2. Stem:

The stem is circular in cross sectional view. It includes thick superficial periderm, thick cortex, narrow phloem cylinder and dense solid xylem cylinder (Fig. 3.3 and 3.4). The periderm is enclosed within an intact darkly stained epidermal cell. The periderm is 100µm thick. It includes about eight layers of tabular, homocellular suberized cells arranged in thick radial row. The cortex is wide and includes large, thick walled less compact parenchyma cells of various shape and size. Secondary phloem occurs in continuous circle around the xylem cylinder. It includes small clusters of sieve elements mixed with phloem parenchyma. The sieve elements have distinct companion cells (Fig. 3.5). Secondary xylem is compact dense cylinder and includes vessels, xylem fibres and xylem rays. The vessels are fairly wide and circular or narrow and not very distinct from wide fibres. The vessel walls are thick and lignified. The fibres are highly thick walled with wide lumen and the xylem rays are prominent and occur in straight prominent lines. The ray cells are also wide, thick walled and lignified. The vessels are 20µm in diameter and the narrow vessels are 10µm wide (Fig. 3.6).
1.4.1.3. Root:

Both thin and thick roots were studied.

Thin root:

A thin root measuring about 2.35mm thick was studied (Fig. 4.1). The thin root is circular in cross section with undulate outline. The epidermis and a few cortical cells are crushed forming dark surface layer. The cortical zone is wide and the cortical cells are large, lobed and compact (Fig. 4.2). The secondary phloem consists of a thin continuous layer running around the xylem cylinder (Fig 4.2). The xylem cylinder is 120µm in diameter and it consists of scattered, solitary, circular vessels which are thick walled (Fig. 4.2). The vessels are upto 25µm in diameter. The fibres are angular, thick walled and lignified. The fibres occur in compact regular radial rows. The fibre walls are thick and lignified and the lumen is fairly wide. The xylem rays are straight and narrow and their cells are also lignified.

Thick root:

The thick root is highly wavy in outline due to the presence of thick and wide fissures. The thick root is 4mm thick (Fig. 5.1). The root consists of very thick periderm which is deeply folded and grooved. The periderm is differentiated into outer thick wavy cylinder of suberized phellem cells and inner equally thick phelloderm layers. The phelloderm cells are squarish in shape with thin walls and deeply stained. The cortical zone is very prominent and thick. The cells are parenchymatous variously shaped and compact (Fig. 5.2). The secondary phloem is quite thick and surrounds the xylem cylinder all around. It consists of collapsed outer phloem and inner narrow non collapsed phloem (Fig. 5.3). In the collapsed phloem the parenchyma cells and phloem rays are dilated and the sieve elements are crushed into dark tangential lines (Fig. 5.4). The non collapsed phloem is very narrow and
includes a few layers of intact sieve elements. In the phloem parenchyma cells have dense accumulation of starch grains (Fig. 5.6). The starch grains are also seen in the cortical parenchyma.

The secondary xylem is circular, dense and compact. It includes circular lines of narrow, thick walled, solitary vessels and thick walled lignified compact radial lines of xylem fibres. The vessels are narrow or wide. The vessel elements are upto 30µm in diameter (Fig. 5.3 and 5.5).

1.4.1.4. Powder microscopy:

The powder preparation of the material exhibits the following inclusions:

I) Fragments of adaxial epidermis were seen in surface view. The epidermis is apostomatic. The epidermal cells are small, and their anticlinal walls are thick and highly wavy (Fig. 6.1).

II) The abaxial epidermal peeling was also seen in the powder. The epidermis is stomatiferous. The stomata are cyclocytic type. A stoma is surrounded by two polar subsidiary cells and two lateral subsidiary cells. Some of the stomata have more than four subsidiary cells which encircle the guard cells (Fig. 6.2 and 6.3). The anticlinal walls of the epidermal cells are thick and wavy. The guard cells are 15×20µm in size.

III) Foliar sclereids: Long, filiform, branched thread like foliar sclereids are wide spread in the lamina. The sclereids run along with the veins and at certain places they come out of the veins and penetrate into the vein islets (Fig. 6.4, 6.5, 7.1 and 7.2). The sclereids are 10µm thick and unlimited in length.

IV) Epidermal trichomes: Epidermal trichomes are seen either attached on the lamina or free from the lamina (Fig. 6.5 and 6.6). The trichomes are
 unicellular, unbranched, pointed at the tip and highly thick walled. The trichomes are nonglandular type. The trichome measures 400µm long and 20µm thick.

V) Xylem fibres: Xylem fibres are abundant in the powder. The fibres are narrow, thick walled and tapering at the end. The cell lumen is fairly wide. No pits are evident. The fibres are upto 550µm long and 15µm thick (Fig. 7.4, 7.5 and 7.7).

VI) Vessel elements: Vessel elements are equally abundant in the powder. Most of the vessel elements are long, narrow and fibre like in appearance. They have simple, oblique elliptical perforations at the end. The pits are circular, multiseriate and bordered. Some of the vessel elements have long narrow tails (Fig. 7.3, 7.4, 7.5 and 7.6).

VII) Epidermal cells: The epidermal cells of the stem were seen in surface view. The cells are vertically elongated, rectangular and run parallel to each other. The cells have thick walls and circular wide simple pits (Fig. 7.8).

1.4.2. WALSURA TRIFOLIATA (A. Juss.) Harms.

1.4.2.1. Leaf:

The leaf in sectional view exhibits smooth adaxial surface and prominent midrib with adaxial concavity (Fig. 9.1). The midrib is concavo-convex in sectional view, having wide shallow adaxial concavity and thick convex on the abaxial side. The midrib 500µm in vertical section and 500µm in horizontal plane; it is 450µm in vertical plane (Fig. 9.2).

The epidermal layer of the midrib consists of small, highly thick walled squarish cells with thick cuticle. The ground tissue includes angular, thin walled
compact parenchyma cells; some of the ground parenchyma cells possess dense accumulation of tannin (Fig. 9.2).

The vascular system includes two circular masses of collateral strands located in the adaxial side and one wide bowl shaped abaxial strand (Fig. 9.3). Both adaxial and abaxial strands are collateral and their xylem strands are just opposed. Phloem occurs on the outer part of the xylem. The xylem elements are wide, elliptical in outline and occur in short or long radial chains. Phloem elements are in small groups, located on their outer part of the xylem mixed with parenchyma cells and fibre sheath. The fibre sheath extends cellular growth on the adaxial and abaxial surfaces of the vascular strands (Fig 9.3).

**Lamina:**

The lamina is dorsiventral with distinct differentiation of the dorsal and ventral surfaces. The adaxial (Ventral) surface of the lamina consists of prominent vertically oblong epidermal cells with thick cuticle. The abaxial epidermis includes small, squarish thick walled cells with finger like epidermal trichome arising from every epidermal cell, the lamina is 260µm thick (Fig. 10.1). The palisade cells occur in one or two compact dense layer of cells on the adaxial side. The spongy mesophyll includes lobed small cells interconnected with each other and forming wide air chambers. Vascular strands are seen in the middle part of the mesophyll.

**Leaf margin:**

As seen in T.S view the leaf margin is slightly bent down and it measures about 200µm thick. The basic structure of the leaf margin is similar to that of lamina region; it includes adaxial palisade zone abaxial reticulate spongy mesophyll tissue and small vascular strand located in the mesophyll tissue. The extreme margin of the lamina includes a small compact mass of thick walled cells (Fig. 10.2)
Epidermal cells and stomata:

The epidermal cells and stomata were studied in surface view of the paradermal sections. The epidermal cells are small polygonal with thick straight anticlinal walls. The stomata are deeply sunken in the epidermal layer. The guard cells are surrounded by 9 to 11 radiating subsidiary cells. Thus the stoma appears to be stellate stomata (Fig. 10.3, 10.4 and 10.5). The guard cells are broadly elliptical measuring 20×20µm in size. The stomatal aperture is narrow and slit like (Fig. 10.4 and 10.5).

Venation pattern of the lamina:

The veins and vein lets are thick and straight. They form fairly wide vein islets with well defined thick and straight vein boundaries (Fig. 10.6). Almost all vein islets have vein terminations. There may be more than one termination in vein islet. The terminations are either unbranched or branched once. They are short and thick and curved (Fig. 10.7).

Petiole:

The petiole is circular with short two lateral wings. It is 1mm thick. The petiole consists of thin epidermal layer which is often broken due to growth in diameter of the petiole (Fig.11.1). There is a narrow less prominent periderm. The cortical zone is parenchymatous the cell being small and circular. The inner boundary of the cortex is marked by a thin cylinder of discontinuous masses of fibres. The vascular cylinder consists of outer wide cylinder of xylem and phloem and central mass of tangentially oblong vascular bundle. The outer cylinder includes outer thick cylinder of secondary phloem in which the phloem elements occur in radial compact files. The secondary xylem includes several radial lines of xylem elements and thick walled xylem fibres. The central strand consists of a few compact lines of xylem
elements mixed with phloem fibres. The phloem elements occur in the form of wide hollow cup on the lower arc of the xylem strand (Fig. 11.2).

1.4.2.2. Stem:

The stem is circular in outline with epidermis, cortex, sclerenchyma cylinder, secondary phloem and secondary xylem. The stem is 2.35mm thick (Fig. 11.3). The stem consists of an epidermal layer which has undergone periclinal divisions producing narrow periderm zone. The cells of the epidermis have thick walls with spiny cuticle. The periderm cells are in 4 or 5 layers of rectangular cells and suberized. Inner to the periderm is wide cortex which includes small, compact parenchyma cells of various shape and size. The cortical zone is about 50µm in thickness. The boundary layer of the cortex is marked by a few isolated irregular masses of sclerenchymatous cells (Fig. 11.4). The secondary phloem quite thick comprises two or three layers of phloem sclerenchyma alternating phloem elements. The outer part of the secondary xylem includes collapsed sieve elements and inner part includes non collapsed intact sieve elements. Secondary xylem cylinder is circular and lobed. It includes several radial lines of vessels and xylem fibres. Xylem rays are well marked and they are thin and straight, running from secondary xylem to secondary phloem. The vessels are circular and the secondary xylem vessels are upto 30µm wide. The pith includes both thin walled parenchyma and thick walled fibres. These two types of cells are mixed with each other (Fig 11.5).

Bark:

T.S of bark of the stem:

The surface of the stem bark is highly fissured with remnants of peeling of surface fissures. There are deep and wide fissures left by scaling off the periderm (Fig. 12.1). The periderm seems to be deeper in origin arising from several layers of
inner secondary phloem. The periderm includes larger portion of inner periderm which has arisen from outer part of the secondary phloem. This periderm consists of several radial files of rectangular thin walled suberized phellem cells. Inner to this periderm zone is the collapsed phloem which consists of several tangential bands of sclerenchyma segments alternating with collapsed phloem tissue (Fig. 13.1 and 13.2). The rays are also dilated in the collapsed phloem zone (Fig. 12.2). The non collapsed phloem is sharply distinguished from the collapsed phloem by the absence of wide dilated rays and intact sieve elements. In the non collapsed phloem the phloem fibres are seen in several tangential blocks alternating with non collapsed phloem cells (Fig. 13.3 and 13.4). The phloem rays are narrow, and do not expand crushing the sieve elements. In the non collapsed phloem the sieve elements are located in radial lines and the cells are rectangular with lateral companion cells (Fig. 13.4).

Calcium oxalate crystals are abundant along the boundaries of sclerenchyma segment (Fig. 13.5 and 13.6).

**TLS view of the phloem:**

The phloem rays are non-storied. They are long and spindle shaped. The ends of the rays are located at different levels. The individual rays are multiseriate, biseriate or uniseriate. The multiseriate rays are heterocellular and they possess middle squarish cells and terminal upright cells. The end wall plate of sieve element is simple and vertically oriented (Fig. 14.1 and 14.2). Calcium oxalate prismatic crystals are abundant in the phloem rays and phloem parenchyma. They are seen arranged in continuous vertical rows. The crystals are rhomboidal or prismatic type (Fig 14.3 and 14.4).
1.4.2.3. Powder microscopy:

The powder preparation of the material shows epidermal peeling in surface view. The adaxial surface of the epidermis exhibits square shaped cells which are compact two or three layered and wide circular simple pits are seen on the walls of the epidermal cells (Fig. 15.1 and 15.2)

Vessels and fibres are abundant in the powder (Fig 15.3). The fibres are long, thin needle shaped. A fibre is 500µm long and 10µm thick. Some of the fibres have highly reduced lumen and thick secondary walls (Fig 15.4).

Vessel elements:

Long cylindrical thick vessel elements are found in the powder. They have simple slightly oblique end wall perforations (Fig. 15.6). Some of the vessel elements have short tails and well developed pits (Fig. 15.7). The vessel elements are upto 250µm long and 40µm wide.

1.4.3. Results of analytical studies:

The physicochemical parameters of *Naregamia alata* and *Walsura trifoliata* were studied by determining the total ash value, alkalinity, water soluble ash, acid insoluble ash, loss on drying, water, alcohol soluble extractive and successive extractive values in various solvents like hexane, petroleum ether, chloroform and ethanol. The observations are tabulated in Table 1.

The TLC profile of ethanolic extracts of the two plants (Fig. 16) is tabulated in Table 2 and 3. Fluorescence characters of the plants were studied under daylight and ultra violet light (265nm and 365nm) in different treatments such as hexane, petroleum ether, chloroform, ethylacetate, ethylalcohol, distilled water, 1N NaOH and 1N HCl. The observations are tabulated in Table 4.

Qualitative organic contents of the alcoholic extracts were studied and the presence or absence of some organic matters is tabulated in Table 5.
1.5. DISCUSSION

The family Meliaceae contains more than 50 genera and about 500 species all over the world. These are mainly distributed in the tropics and warm temperate regions. In India, this family is represented by 20 genera 113 species (Hooker, 1872).

It is observed that, in *Naregamia alata*, flowers occur in solitary form, the fruits are capsule and petioles are winged. In *Walsura trifoliata*, flowers occur in corymbose panicles and the fruits are berry and the petioles are not winged. These morphological characters play a key role in distinguishing *Naregamia alata* from *Walsura trifoliata*.

While discussing the habit of Meliaceae, Hooker (1872) distinguishes woody species and shrub species. In *Walsura trifoliata*, the upper epidermal cells are vertically oblong with thick cuticle. In shrub species (*Naregamia alata*), epidermal cells are thick with smooth cuticle. Both the species are characterized by cyclocytic stomata.

Of the two species studied during the present investigation the correlation of the epidermal cell walls, parenchyma distribution of the plants could not be satisfactorily explained with their habitat. In *Walsura trifoliata*, which is a tree inhabiting dry-deciduous habitats the anticlinal walls of the epidermis are straight; stomata is sunken; the palisade tissue is 1-2 layered; spongy mesophyll cells are interconnected to one another forming wide airchambers. This feature may be visualized as dry-deciduous environmental impact. In *Naregamia alata*, the palisade layers are short, conical and compact ones. Spongy mesophyll cells are loosely arranged. These characters exhibit mesomorphic features as far as the palisade structures are concerned.
The vascular system in the leaves of Meliaceae differs widely from one another in such details as massiveness, development of parenchyma versus sclerenchyma in the bundle sheath and vein endings. Thus the leaf vascular system appears to be a prominent source of systematically valuable criteria (Webster 1957). In the two species studied adaxial vascular bundle of *Naregamia alata* is small, circular and collateral with prominent sclerenchymatous cap. In *Walsura trifoliata* the midrib bundle is circular, collateral without the sclerenchymatous cap (Fig. 9.3). *Walsura trifoliata* has more distinct vein islets and vein terminations are short, thick and curved. In *Naregamia alata* intra marginal veins and vein islets are thin and less prominent, and vein terminations are long slender and wavy (Fig. 2.6).

The structural diversity of petiolar anatomy and its application in systematics have been recognized by the earlier anatomists. Grew (1675), De Candolle (1879) and Hare (1943) are the pioneers in the study of petiole and have proposed extensive terminology and structural variations of the petiole. The two species of the present study have distinctive structures of the petiole. *Naregamia alata* has two prominent vascular strands, the adaxial side with semicircular and in abaxial side bowl shaped vascular cylinder which is protected by thick arc of lignified fibre. *Walsura trifoliata* has characteristic wide cylinder on adaxial side and abaxial side oblong vascular cylinder. Thus the two species of the present study have distinctive features in the petiole.

Wood anatomy of the two species of the present study has the distinctive anatomical features suitable for the diagnostic purpose. The stem woods of *Naregamia alata* and *Walsura trifoliata* have mostly radial and multiples of vessels.

In *Naregamia alata*, vessels are long, narrow fibre like with oblique elliptical perforations at the end with long narrow tails. The pits are circular, multiseriate. In
Walsura trifoliata, the wood has long, cylindrical, thick vessels. The vessels have simple, slightly oblique end wall perforations with short tail and well developed pits (Fig. 15.6 and 15.7). A comparison of salient features of two taxa is presented in Table 6.

Physicochemical parameters are important in detecting adulteration and are adopted to confirm the purity and quality of the drug.

The present study reveals that the loss on drying does not vary much for the two plants (7.87 and 7.01%w/w) (Table 1). Its dried form is expected to have a long shelf-life with reduced chance of microbial growth due to its relatively low moisture content. Insufficient drying favours spoilage by moulds and bacteria make possible the enzymatic destruction of active principles. It is observed that the drug is properly dried and properly stored.

Total ash value for the two plants (5.82 and 4.97%w/w) indicates low inorganic components in the plants (Table 1). Total ash is particularly important in the evaluation of purity of drugs i.e., the presence or absence of foreign matter such as metallic salts or silica.

The amount of acid insoluble siliceous matter present in the two plants is 1.04 and 0.34%w/w. The water soluble ashes are found to be 1.85 and 1.17%w/w(Table 1). These parameters are used to detect the presence of foreign material exhausted by water. As the ash values of the crude drugs lie within the fair limit which signify its quality and purity and gives idea about the total inorganic content.

The water soluble extractive values are found to be 14.63 and 15.10%w/w (Table 1). The alcohol soluble extractive values for the two plants are 12.31 and 10.09%w/w which indicates the presence of polar constituents like phenols,
glycosides and flavonoids. The water soluble extractive is found to be signifying that the large amounts of constituents of the plants are soluble in water than in alcohol.

Extractive values of the plant with different solvents give a preliminary picture of the percentage of the compounds extracted. Extraction with ethyl alcohol gives the highest yield in the successive extraction (5.4 and 4.18% w/w). So ethyl alcohol is the best solvent for extraction among the five solvents used (Table 1).

In the TLC profile, the extracts of the two plants in ethyl acetate and Toluene (1:1) solvent system confirm the presence of diverse potent biomolecules in these plants. In *Walsura trifoliata* it shows maximum seven bands under UV 366nm (Fig. 16.2). In *Naregamia alata* maximum seven bands are observed with vanillin sulphuric acid spray reagent (Fig. 16.1). TLC analysis provides an idea about the polarity of various chemical constituents, in a way such that compound showing high Rf value has low polarity and with less Rf value has high polarity (Table 2 and 3). These potent biomolecules can be further used for the development of different drugs in future.

Fluorescence analysis is an essential parameter for first line standardization of crude drug. The fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelength is very active in producing fluorescence and for this reason UV light produces fluorescence in many substances which do not visible in day light. Plants usually contain both fluorescent and non-fluorescent compounds. When powdered samples of these plants are exposed to UV light of shorter (265nm) and longer wavelengths (365nm) they emit specific colours depending on the wavelengths of light. However, the colours will be constant for any given extract. This procedure helps to standardize the fluorescent colour characteristic for a drug (Table 4).
For qualitative tests, ethyl alcohol extract was used for the two plants. The extracts were positive for triterpenoid, alkaloid, phenol, flavonoid, coumarin, quinone, furan, tannin and sugars. Steroid and saponins are absent in the two plants under study (Table 5).

Thus, simple chemical analysis of crude drugs of the two plants helps in two ways. It gives a preliminary idea for the prospects of the chemical compounds present in the plants and it gives some chemical parameter for determining the quality and purity of the raw drugs obtained from the market.
TABLE 1

PHYSICO CHEMICAL CONSTANTS

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Naregamia alata value (%w/w) (Whole plant)</th>
<th>Walsura trifoliata value (%w/w) (Aerial parts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total ash value</td>
<td>5.82</td>
<td>4.97</td>
</tr>
<tr>
<td>2.</td>
<td>Water soluble ash</td>
<td>1.85</td>
<td>1.17</td>
</tr>
<tr>
<td>3.</td>
<td>Alkalinity of water soluble ash</td>
<td>0.48ml of 0.1N HCl/g</td>
<td>0.6ml of 0.1N HCl/g</td>
</tr>
<tr>
<td>4.</td>
<td>Acid insoluble ash</td>
<td>1.04</td>
<td>0.34</td>
</tr>
<tr>
<td>5.</td>
<td>Loss on drying at 105°C</td>
<td>7.87</td>
<td>7.01</td>
</tr>
<tr>
<td></td>
<td>Solubility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Water soluble extractive</td>
<td>14.63</td>
<td>15.10</td>
</tr>
<tr>
<td>7.</td>
<td>Alcohol soluble extractive</td>
<td>12.31</td>
<td>10.09</td>
</tr>
<tr>
<td>8.</td>
<td>Extractive value (Successive extraction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Hexane</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>b.</td>
<td>Petroleum ether</td>
<td>2.7</td>
<td>2.52</td>
</tr>
<tr>
<td>c.</td>
<td>Chloroform</td>
<td>3.2</td>
<td>2.73</td>
</tr>
<tr>
<td>d.</td>
<td>Ethyl alcohol</td>
<td>5.4</td>
<td>4.18</td>
</tr>
</tbody>
</table>
TABLE 2
TLC PROFILE OF WHOLE PLANT ETHANOLIC EXTRACT OF NAREGAMIA ALATA

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>UV 254nm</th>
<th>UV 366nm</th>
<th>WITH VANILLIN SULPHURIC ACID SPRAY REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COLOUR</td>
<td>RF</td>
<td>COLOUR</td>
</tr>
<tr>
<td>1.</td>
<td>Green</td>
<td>0.08</td>
<td>Red</td>
</tr>
<tr>
<td>2.</td>
<td>Red</td>
<td>0.10</td>
<td>Red</td>
</tr>
<tr>
<td>3.</td>
<td>Red</td>
<td>0.14</td>
<td>Blue</td>
</tr>
<tr>
<td>4.</td>
<td>Blue</td>
<td>0.44</td>
<td>Red</td>
</tr>
<tr>
<td>5.</td>
<td>Red</td>
<td>0.49</td>
<td>Red</td>
</tr>
<tr>
<td>6.</td>
<td>Red</td>
<td>0.86</td>
<td>Grey</td>
</tr>
<tr>
<td>7.</td>
<td>Red</td>
<td></td>
<td>Grey</td>
</tr>
</tbody>
</table>

TABLE 3
TLC PROFILE OF AERIAL PARTS ETHANOLIC EXTRACT OF WALSURA TRIFOLIATA

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>UV 254nm</th>
<th>UV 366nm</th>
<th>WITH VANILLIN SULPHURIC ACID SPRAY REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COLOUR</td>
<td>RF</td>
<td>COLOUR</td>
</tr>
<tr>
<td>1.</td>
<td>Green</td>
<td>0.05</td>
<td>Red</td>
</tr>
<tr>
<td>2.</td>
<td>Green</td>
<td>0.77</td>
<td>Red</td>
</tr>
<tr>
<td>3.</td>
<td>Blue</td>
<td>0.43</td>
<td>Red</td>
</tr>
<tr>
<td>4.</td>
<td>Red</td>
<td>0.57</td>
<td>Red</td>
</tr>
<tr>
<td>5.</td>
<td>Red</td>
<td>0.76</td>
<td>Grey</td>
</tr>
<tr>
<td>6.</td>
<td>Red</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Red</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 4

**FLUORESCENCE ANALYSIS**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatment</th>
<th><em>Naregamia alata</em> (Whole plant)</th>
<th><em>Walsura trifoliata</em> (Aerial parts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Under ordinary light</td>
<td>Under UV light (265nm)</td>
</tr>
<tr>
<td>1.</td>
<td>Powder</td>
<td>Green</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>2.</td>
<td>Powder+Petroleumether</td>
<td>Pale yellow</td>
<td>Green</td>
</tr>
<tr>
<td>3.</td>
<td>Powder+Hexane</td>
<td>Yellowish green</td>
<td>Green</td>
</tr>
<tr>
<td>4.</td>
<td>Powder+Chloroform</td>
<td>Yellowish green</td>
<td>Light green</td>
</tr>
<tr>
<td>5.</td>
<td>Powder+Ethylacetate</td>
<td>Dark yellow</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>6.</td>
<td>Powder+Ethyl alcohol</td>
<td>Yellow</td>
<td>Pale green</td>
</tr>
<tr>
<td>8.</td>
<td>Powder+1N NaOH</td>
<td>Yellow</td>
<td>Light green</td>
</tr>
<tr>
<td>9.</td>
<td>Powder+1N HCL</td>
<td>Pale yellow</td>
<td>Pale green</td>
</tr>
</tbody>
</table>
### TABLE 5

**QUALITATIVE ORGANIC ANALYSIS**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Contents of the alcoholic extract</th>
<th><em>Naregamia alata</em> (Whole plant)</th>
<th><em>Walsura trifoliata</em> (Aerial parts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Steroid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Triterpenoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Phenol</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Coumarin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Quinone</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Furan</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Glucoside/Sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Saponin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.NO</td>
<td>CHARACTERS</td>
<td>NAREGAMIA ALATA</td>
<td>WALSURA TRIFOLIATA</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
<td>----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Habit</td>
<td>Under shrub</td>
<td>Tree</td>
</tr>
<tr>
<td>2.</td>
<td>Habitat</td>
<td>Moist deciduous forests</td>
<td>Dry ever green forests</td>
</tr>
<tr>
<td>3.</td>
<td>Leaf</td>
<td>Trifoliate, cuneate-obovate</td>
<td>Trifoliate, elliptic</td>
</tr>
<tr>
<td>4.</td>
<td>Fruits</td>
<td>Capsule</td>
<td>Berry</td>
</tr>
<tr>
<td>5.</td>
<td>Stomatal type</td>
<td>Cyclocytic stomata</td>
<td>Cyclocytic stomata</td>
</tr>
<tr>
<td>6.</td>
<td>Subsidiary cells</td>
<td>Six or more</td>
<td>Nine to eleven</td>
</tr>
<tr>
<td>7.</td>
<td>Guard cells</td>
<td>15×20µm in size</td>
<td>20×20µm in size</td>
</tr>
<tr>
<td>8.</td>
<td>Lamina</td>
<td>120µm thick</td>
<td>260µm thick</td>
</tr>
<tr>
<td>9.</td>
<td>Veinlets</td>
<td>Thin and less prominent</td>
<td>Thick and straight</td>
</tr>
<tr>
<td>10.</td>
<td>Vein terminations</td>
<td>Long, slender and wavy. Either unbranched or branched once or twice</td>
<td>Short, thick and curved. Either unbranched or branched once</td>
</tr>
<tr>
<td>11.</td>
<td>Petiole</td>
<td>Two lateral wide wings</td>
<td>Two lateral short wings</td>
</tr>
<tr>
<td>12.</td>
<td>Stem</td>
<td>Occurs in continuous circle around the xylem cylinder.</td>
<td>Quite thick, comprising 2 or 3 layers of phloem sclerenchyma, alternating phloem elements.</td>
</tr>
<tr>
<td>13.</td>
<td>Periderm</td>
<td>Eight layers of tabular, suberized cells</td>
<td>Four or five layers of rectangular, suberized cells</td>
</tr>
<tr>
<td>14.</td>
<td>Powder microscopy Fibres</td>
<td>Abundant, narrow, thick walled and tapering at the end, upto 550µm long and 15µm thick.</td>
<td>Equally abundant, long, thin, needle shaped upto 500µm long and 10µm thick.</td>
</tr>
<tr>
<td>15.</td>
<td>Vessel elements</td>
<td>Abundant, long, narrow and fibre like, they have simple, oblique, elliptical perforations at the end, long narrow tails. Pits are circular, multiseriate and bordered.</td>
<td>Abundant, long, cylindrical, thick, they have simple slightly oblique end wall perforations, short tails and well developed pits.</td>
</tr>
<tr>
<td>17.</td>
<td>Spiny cuticle</td>
<td>Present in the petiole epidermis.</td>
<td>Present in the stem epidermis.</td>
</tr>
</tbody>
</table>