

Pharmacognostical Studies on *Salmalia insignis* (Wall.) Schott. & Endl., A Bombacaceae Member

Sharmila S^{1*}, Mownika S, Ramya E K

PG and Research Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode – 638012, Tamil Nadu, India.

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ABSTRACT

The macroscopic, microscopic, histological identification and microscopic constants of *Salmalia insignis* can be used as a rapid, inexpensive and botanical identification technique which would be of immense value in standardization and authentication of this plant. The present study was carried out to investigate the preliminary phytochemical screening and in vitro antioxidant activity of *Salmalia insignis*. In the present work we investigated the phytochemical screening to find out new sources of natural antioxidant activity source from the leaf of *Salmalia insignis* with different solvents such as ethanol and petroleum ether. The phytochemical screening was carried out using standard phytochemical tests. Quantitative analysis was performed to confirm and quantify the presence of phenolics content and total flavonoids in the aerial plant extracts of the study plant. The in vitro antioxidant activity was tested on Hydroxyl radical scavenging activity of different solvents such as Petroleum ether, Ethanol and Butylated hydroxynisole. These compounds correspond to varied medicinal properties that can be exploited for the treatment of many diseases.

Keywords: *Salmalia*, phytochemical, Butylated hydroxynisole and Hydroxyl radical scavenging activity.

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Martins, 2001). Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh *et al.*, 2004).

Over the last decade, due to the limitations associated with synthetic pharmaceutical products; the avenues have been opened for Green medicine which is considered to be safe, more accessible and affordable too (Mithraja *et al.*, 2011). Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity forms an essential aspect of its study. It becomes extremely important to make an effort towards standardization of the plant material. The process of standardization can be achieved by stepwise pharmacognostic studies. This study helps in identification and authentication of plant material. It is estimated that

about 25% of all modern medicine are directly or indirectly derived from higher plants.

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow *et al.*, 2003). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against harmful diseases (Benkeblia, 2004). Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh *et al.*, 2004).

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Plate 1: Habit of *Salmalia insignis*.

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Nowadays herbal drugs are in great demand than ever before. The public were also of general awareness in regarding the safety and efficacy of herbal drugs. Quality control studies on plant material are essential to ensure the reproducible quality of the herbal products. The initial step to ensure quality of any starting material is authentication (Lalithrani *et al.*, 2011). WHO suggests that the macroscopic and microscopic description of a plant material is the primary step in establishing its identity (Anonymous, 1998). Standardization of herbal medicines and quality control of the plant raw materials are very important aspects of manufacture and supply of herbal drugs. Therefore the macroscopic characterization is used to establish pharmacognostic profile, which will help in crude drug identification as well as in standardization of the quality and purity.

Pharmacognosy means knowledge of drugs which is mainly concerned with naturally occurring substances. This deals with biological, biochemical, therapeutic and economic features of natural drugs and their chemical constituents (Mohammed Ali, 2007). In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.*, 2005). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (Tanaka *et al.*, 2002).

Bombacaceae is a small family of the order Malvales and contains about 28 genera and 200 species. Members of this family are not only showy ornamentals but they possess

significant economical and commercial reputation as well. In addition, various plant parts of several species are widely used as foods and traditional medicines in many parts of the world. *Salmalia insignis* (Wall.) Schott. & Endl. Is one of the important genera of the family Bombacaceae distributed all over the plains of India. They are native to western Africa, the Indian subcontinent, Southeast Asia, as well as subtropical regions of East Asia and northern Australia. The species name *insigne* means striking, noted, spectacular. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani.

Bombax ceiba is used in Ayurvedic medicine for the treatment of aphrodisiac, astringent, antidiarrheal, antidysenteric, antimicrobial diuretic, alterative, antipyretic and tonic. *Salmalia insignis* has got strong ethno-pharmacological background, useful in the treatment of diarrhoea and antibacterial activity. No detailed pharmacognostic and pharmacological studies or establishment of quality parameters has been done on any species of *Bombax*. The present investigation deals with the macroscopic, microscopic and histochemical localization of leaf and stem and its quality parameters, including physicochemical and phytochemical evaluation. Mainly the study was focused to analyse the micro morphological features of this drug source and to localize the active molecules histochemically.

MATERIALS AND METHODS

Study area

Fresh leaves of *Salmalia insignis* were collected from, ABS Botanical Gardens, Kaaripatti, Salem. It is rich in Biodiversity and indigenous population. They are conserving more than 2500 plant species including rare and endangered medicinal plants. The area of investigation approximately lies 11° - 39' latitude and 78° - 17' longitude.

Plant collection

The plants were collected in their flowering and fruiting seasons from the natural habitat. While collecting the study plant, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics etc.

Plant identification

The collected study plant was identified with the help of the existing Floras (Bor, 1986; Dietrich brandis, 1978; Fyson, 1915-20; Matthew, 1983) and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu and ABS Botanical conservation, Research & Training Centre (voucher specimen number - AUT/VCW/056). The collected plant specimens were pressed properly following the method of Jain and Rao (1970). Dried specimens were poisoned with 0.1% HgCl₂ dissolved in absolute alcohol and mounted with glue on standard herbarium sheet (42 x 28 cm). The herbaria were deposited in Department of Botany, Vellalar College for Woman, Thindal, Erode. Photographs were also taken to supplement the herbarium (Plate - 1).

Plant taxonomy

Taxonomical studies on the plant have been carried out and its systematic position was assigned as per the angiosperm taxonomic classification of Bentham and Hooker's (1862-1883).

Systematic Position

Division: Angiospermae
 Class: Dicotyledones
 Sub-Class: Polypetalae
 Series: Thalamiflorae
 Cohort: Malvales
 Family: Malvaceae
 Sub-family: Bombacaceae
 Genus: *Salmalia*
 Species: *insignis*

Vernacular Names

Tamil : Mullilavu
 Malayalam: Kallilavu
 Bengali: Semul-tula
 Sanskrit: Shalmali
 Andamans: Didu
 Gujarati: Shemlo

Distribution

Salmalia insignis (Wall.) Schott. & Endl. is one of the important genera of the family Bombacaceae distributed all over the India. They are native to western Africa, the Indian subcontinent, Southeast Asia, as well as subtropical regions of East Asia and northern Australia. The genus *Bombax* is the largest within the family and has about 60 species. The earlier name of the silk cotton tree was *Salmalia*, derived from its Sanskrit name 'Shālmali'.

Pharmacognostical Studies

Macroscopical studies

Macroscopic characters of *Salmalia insignis* were studied. The morphological characters of the stem and leaf such as colour, surface texture, taste and odour were examined as per Trease and Evans (1983) and Wallis (1985).

Microscopical studies

The leaf and stem of *Salmalia insignis*, were cut and removed from the plant and fixed in FAA (Formalin - 5 ml + Acetic acid - 5 ml + 70% Ethyl alcohol - 90 ml). Thin, free hand transverse sections were made with the help of sharp blade and cleared with chloral hydrate solution. The suitable thin sections were stained with Safranin and mounted in glycerin. The images were recorded on a Photonic microscope (Model A X 70 TRF, Olympus optical) with a camera. The vascular bundle arrangement and histology of leaves and stems were classified according to Metcalf and Chalk, 1979. Leaf constants were established according to Anonymous, 1988.

Histochemical studies

Micromorphological characterization studies were carried out employing standard sectioning and staining methods as per standard texts. The cell arrangements, size and shape of cells, starch grains and stomatal types were localized histochemically in the leaf and stem of study plant using specific reagents and were recorded on a Photonic microscope (Model A X 70 TRF, Olympus optical). Fresh free hand sections of leaf and stem of *Salmalia insignis* were taken. The fresh sections of the plant (leaf and stem) used for the histochemical study were treated with the

respective reagents to localize the presence or absence of metabolites. The reagents used were: Mayer's reagents to detect alkaloids, Potassium iodide for tannin, 25% Lead acetate for detection of Flavonoids, Lugol's iodine solution for starch (Jensen, 1962), Sudan-I for total lipids (Brundeff et al., 1991) and 5% Ferric chloride anhydrous for Phenolic compounds. The thin sections were stained with above reagents and the images were recorded on a Photonic microscope (Model A X 70 TRF, Olympus optical) with a camera. Presence of active constituents was confirmed through color development due to the reaction of the cells with specific reagents. Histochemical observations can help in identifying the major chemical constituents. This can be further confirmed by carrying out qualitative tests. These data can contribute in determining the standards for the plant drug under study and can contribute to the Ayurvedic Pharmacopeia of India.

Shade drying and powdering of the collected plant material

Freshly collected leaves were cleaned to remove adhering dust and then shade dried. The shade dried plant materials were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for physicochemical, phytochemical and fluorescence analysis. Samples were stored in the good grade plastic containers which are maintained at room temperature until analysis (Harborne, 1973).

Soxhlet extraction

Dried and powdered leaf powder (50 g) of *Salmalia insignis* was filled in the thimble and extracted successively with petroleum ether and ethanol (50g/250 ml) using a Soxhlet extractor for 8-10 hrs (Plate 2). Extracts thus obtained will be concentrated in rotavapour and separated in glass vials and stored at 4°C in refrigerator for further use. Different dilutions of the extracts will be prepared in DMSO (Di Methyl Sulphoxide). The extracts were subjected to phytochemical analysis and antioxidant activity.

Physico-Chemical Studies

Organoleptic characters of plant powder and the extract

The organoleptic evaluation of aerial plant powder and the extracts, such as colour, texture, odour and taste were carried out as per Trease and Evans (1983).

Behaviour of plant powder with different chemical reagents

The behaviour of powdered plant material treated with different chemical reagents such as concentrated Concentrated HCl, H₂SO₄, acetic acid and ethanol was observed (Kokoshi et al, 1958).

Qualitative Phytochemical Analysis

Phytochemical screening of different successive solvent extracts was carried out following the methods of Horborne (1984) and Kokate et al. (1995). Alkaloids, flavonoids, glycosides, phenols, tannins, saponins, anthroquinone, terpenoids, coumarin, quinone, gum and mucilage, carbohydrate, proteins and amino acids and fixed oil were qualitatively analyzed.

Tests for alkaloids

Wagner's reagent: To 1 ml of the extract, a few drops of Wagner's reagent was added and the formation of a

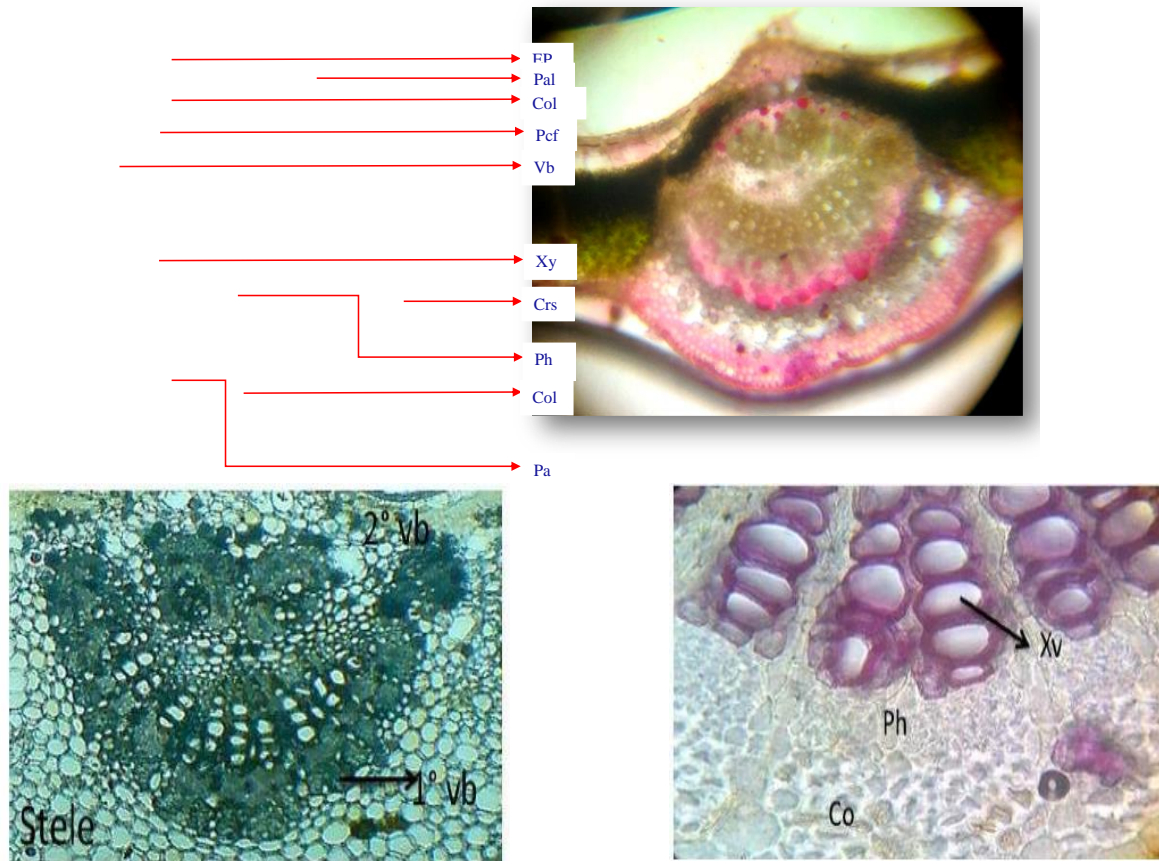


Plate 2: T.S of leaf through midrib-salmalia in signis, T.S of midrib - Enlarged

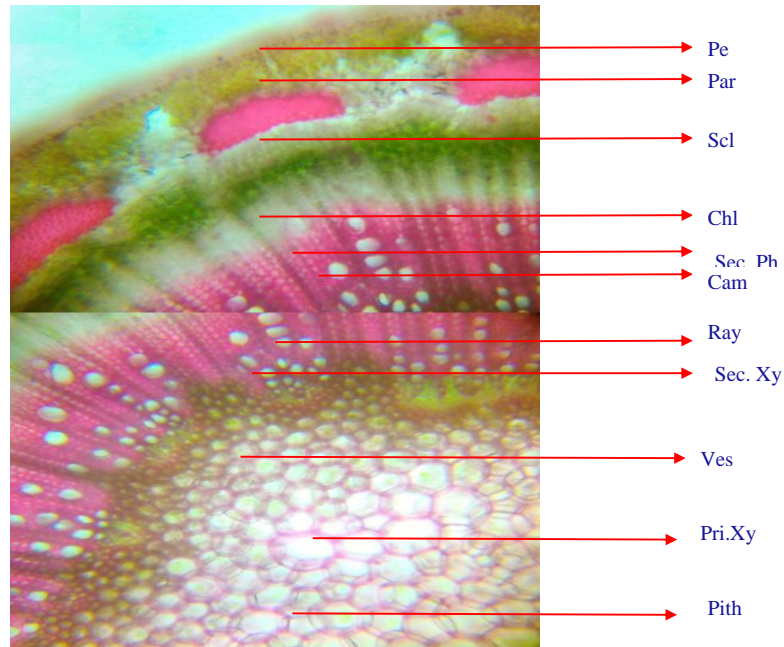


Plate 3: T.S of Stem – *Salmalia insignis*

reddish brown precipitate indicates the presence of alkaloids.

Tests for flavonoids

To 1 ml of the extract, 1 ml of neutral ferric chloride was added. Appearance of brown colour confirms the presence of flavonoids.

Tests for glycosides

Legal test: The extract was dissolved in pyridine and to this freshly prepared sodium nitro prusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

Phenols

Lead acetate test: To 5 ml of extract, 5 ml of distilled water and 3 ml of 10 % lead acetate solution was added. The formation of milky white precipitate confirms the presence of phenols.

Tannins

Ferric chloride test: To 5 ml of extract, 5 ml of distilled water and few drops of neutral ferric chloride solution was added. Appearance of bluish green colour indicates the presence of tannins.

Test for saponins (Foam test)

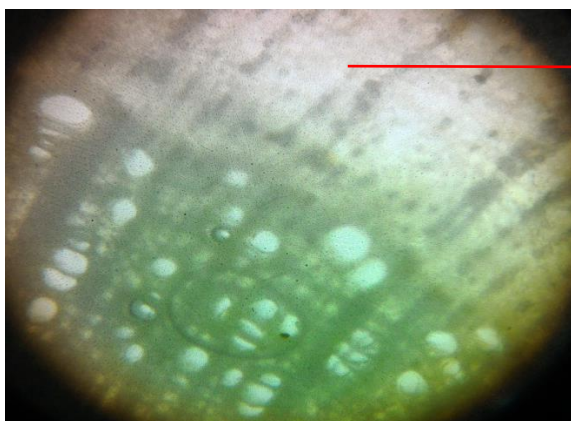
About 1 ml of alcoholic extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicates the presence of saponins.

Anthroquinones

To 0.5 ml of extract, few drops of 2% Hcl was added. Appearance of reddish colour precipitate indicates the presence of anthroquinones.

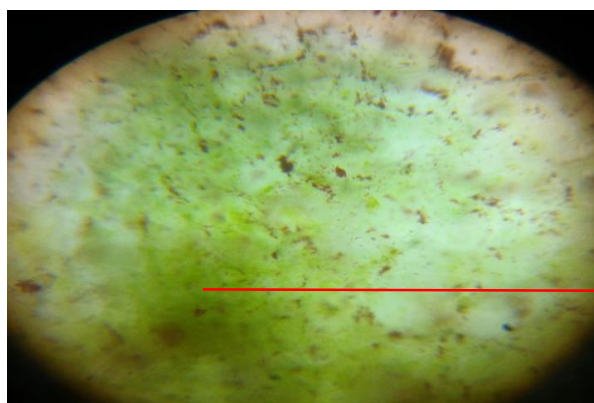
Terpenoides

To 0.5 ml of extract, 2 ml of chloroform and Con. H₂SO₄ was added. Appearance of reddish brown at the interface



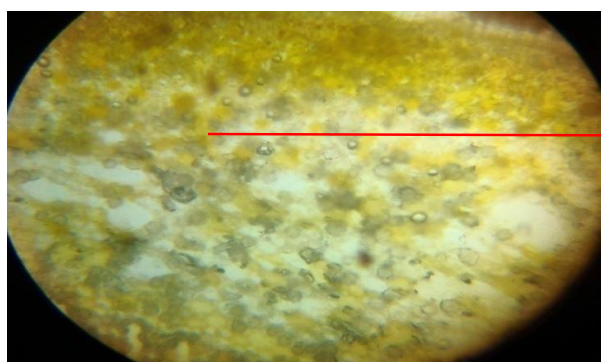
Alkaloids

a) Localization of Alkaloids



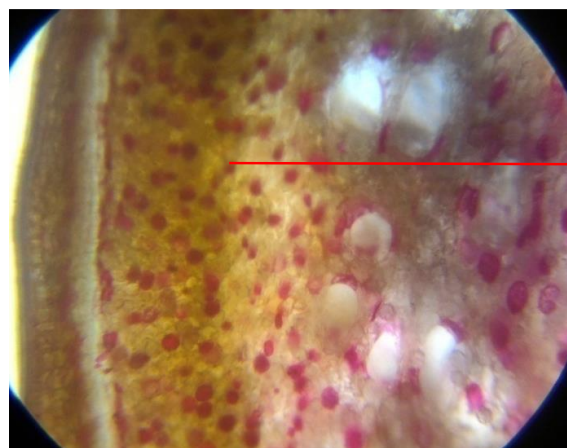
Tannin

b) Localization of Tannin



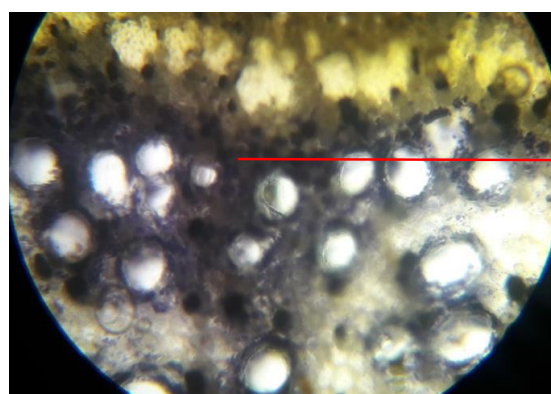
Flavonoids

c) Localization of Flavonoids



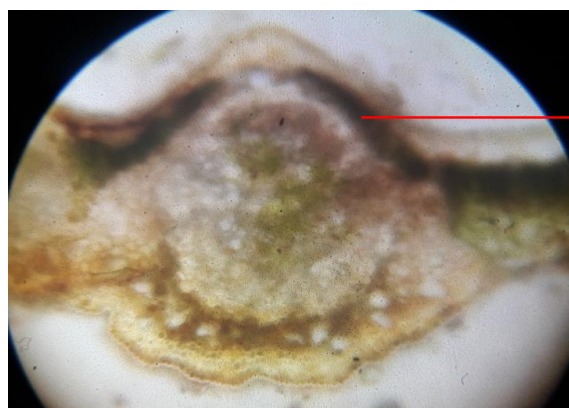
Starch

d) Localization of Starch



Lipid

e) Localization of Lipid



Phenol

f) Localization of Phenol

Plate 4: Histochemical localization of Stem and Leaf

time confirms the presence of terpenoides.

Coumarin

To 1 ml of extract, 1 ml of 10 % Sodium hydroxide was added. Appearance of yellow colour indicates the presence of coumarin.

Quinone

To 1 ml of extract, 1 ml of Con. H_2SO_4 was added. Appearance of red colour indicates the presence of quinone.

Gum and mucilage

To 5 ml of extract, 5 ml of distilled water and 25 ml of absolute alcohol was added on constant stirring. The formation of white or cloudy precipitate confirms the presence of gum and mucilage.

Carbohydrate

Barfoed's test: To 1 ml of extract, 1 ml of Barfoed's reagent was added and heated on a boiling waterbath for 2 minutes. The formation of red precipitate confirms the presence of carbohydrate.

Proteins and Amino acids

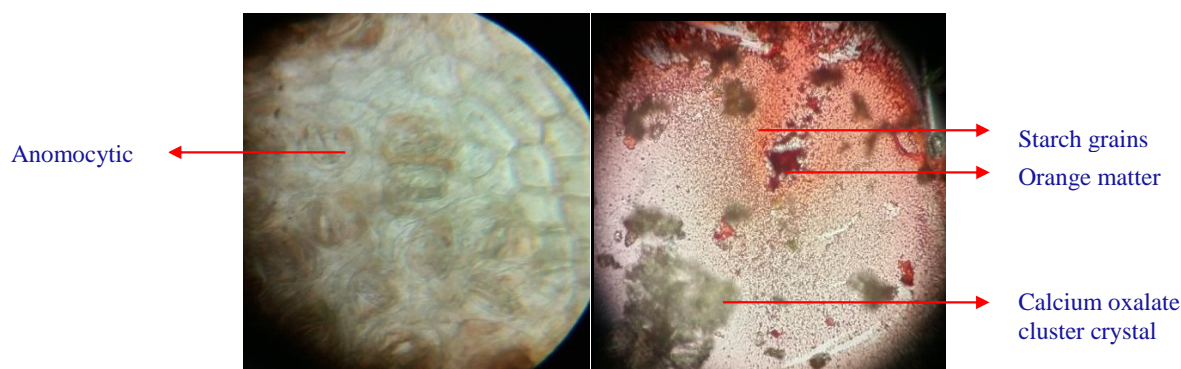


Plate 5: Type of Stomata and powder study

Table 1: Organoleptic characters of plant powder of *Salmalia insignis*.

S.No.	Characters	Observations
1.	Colour	Yellowish Green
2.	Texture	Fine smooth powder
3.	Taste	Bitter
4.	Odour	Characteristic smell

Table 2: Organoleptic characters of plant successive extracts of *Salmalia insignis*.

S. No.	Extraction Medium	Colour	Consistency	Odour
1.	Petroleum ether	Greenish yellow	Semi solid	Characteristic smell
2.	Ethanol	Blackish green	Semi solid	Characteristic smell

Table 3: Behaviour of plant powder of *Salmalia insignis* with different chemical reagents

S. No.	Powder + Reagents used	Colour of the powder
1.	Powder as such	Yellowish Green
2.	Powder + Iodine solution	Pale brown
3.	Powder + Sodium nitroprusside	Yellowish brown
4.	Powder + Lead solution	Pale green
5.	Powder + Antimonytrichloride	Pale yellow

Biuret test: To 2 ml of extract, few drops of 2 % copper sulphate solution and 1 ml of ethanol (95 %) was added. To this potassium hydroxide pellets (excess) was added. The formation of pink colour in ethanol layer confirms the presence of proteins and amino acids.

Fixed oil

Press small quantity of extract between two filter papers. Oil stain on the paper indicates the presence of oil content.

Quantitative Phytochemical Studies

Determination of total phenolics (Siddhuraju and Becker, 2003)

Ten microlitre aliquots of the extracts (10 mg / 2 ml) were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu phenol reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Determination of total flavonoid contents (Zhishen et al., 1999)

0.5 ml aliquot of appropriately (10 mg / 2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% NaNO₂ solution. After 6 minutes, 0.15 ml of 10% AlCl₃ solution was added and allowed to stand for 6 min and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The results were expressed as rutin equivalent.

Antioxidant Activity

Hydroxyl radical scavenging activity (Klein et al., 1991)

Various quantities of extracts (250–1250 µg) were added with 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018%) and 1 ml of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and raised to 1 litre with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectroscopically at 412 nm against reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by the following formula

$$\text{HRSA \%} = 1 - (\text{difference in absorbance of sample} / \text{difference of blank}) \times 100$$

RESULTS

Macroscopical studies

Table 4: Qualitative phytochemical screening of leaf powder extracts of *Salmalia insignis*.

S.No.	Constituents	Petroleum ether extract	Ethanol extract
1.	Akaloid (wayers reagent)	+	-
2.	Flavonoid	-	+
3.	Glycosides	-	-
4.	Phenols	+	-
5.	Tannins	-	+
6.	Saponin	-	-
7.	Anthroquinone	-	-
8.	Terpenoides	-	+
9.	Coumarin	+	+
10.	Quinone	-	+
11.	Gum and mucilage	+	-
12.	Carbohydrate	-	-
	Proteins and		
13.	Amino acids (Nin – hydrin test)	-	-
14.	Fixed oil	-	+

Note : '+', '-' indicates the presence / absence of compounds

The present macroscopical investigations of *Salmalia insignis* revealed that the species is large deciduous tree, reaching 30 to 40 metres in height and up to three metres trunk diameter (Plate-1). The fresh leaf is green in color with characteristic odour and slight bitter taste. The leaves are compound, pinnately trifoliate with 7-9 leaflets arranged on a common petiole, 7 to 10 inches long with entire margins and deciduous, being shed in the dry-season. They measure 30 to 50 cm across and are palmate in shape with five to nine leaflets 1-1.75 inches, obovate in shape, with a pointed tip. Flowers are very showy, scarlet, pink, and creamy or white, clustered towards the ends of the branch lets. Sepal tube is urn-shaped, slightly 2-lobed, 1.5 inches long, sometimes prickly outside, densely silky within (Table 1). Fruit is a capsule reaching almost a foot in length by 2-2.5 inches in diameter, distinctly five-angled (important distinguishing character), filled with silky cotton and round seeds.

Microscopical studies

Leaf

Surface preparation shows the presence of thick walled epidermal cells and anomocytic stomata (Plate 2; Plate 5). Transverse section of the leaf shows a thick midrib, lateral veins and thin dorsiventral lamina having a single layer of compact rectangular epidermal cells with a thick cuticle. Beneath it is a single, compact layer of radially elongated palisade cells are present followed by loosely arranged spongy mesophyll cells rich in starch grains. The mesophyll covers 3/4th of the lamina. Mid-rib consists of well-developed collenchymas cells, present just below the upper epidermis and above the lower epidermis. A sheath of calcium oxalate cluster crystals is present above the lower epidermis in the lower collenchyma of the mid-rib. Ground tissue consists of loosely arranged polygonal parenchymatous cells having orange colouring matter.

Vascular bundles are bicollateral. Primary vascular bundles showed 14 - 15 seriate xylem vessels whereas secondary vascular bundle showed 4 - 5 seriate xylem vessels. Bunch of pericyclicfibres are situated above the secondary vascular bundles, whereas they are discontinuous below the primary vascular bundles.

Young stem

The young stem is 1.9 mm thick. It shows initial stage of secondary growth. The transverse section of *Salmalia insignis* stem shows the outermost layer is periderm which is thin continuous all around the stem; the cells are thin walled, cylindrical in shape. The cortical region is made up of parenchyma cells, patches of sclerenchyma cells and a layer of chlorenchyma cells. Just below the cortical region secondary phloem is present in the form of bast fibers followed by cambium. Below this, inner secondary xylem is seen and it is made up of tracheids, vessels and xylem parenchyma, which is differentiated into autumn wood and spring wood later form annual rings. Patches of primary xylem is present and it is endarch. The centre wood is porous, growth ring boundaries are fairly distinct and marked by denser fiber zones. Fibers are arranged alternately with the parenchyma strand. In the centre large pith is present (Plate 3).

Histochemical studies

In recently, pharmacognogist have started (Plate 4, a-f) histochemical features in solving taxonomic problems and also the identification and characterization of plant drug. The present study screening the presence of alkaloids, flavonoids, tannins, starch, lipids and phenols in leaf and stem of the *Salmalia insignis* which could be used as a diagnostic tool for this plant drug. The presence of alkaloids, flavonoids and phenols were confirmed through color development due to the reaction of the cells with specific reagents provided chemical markers that could be used in identification.

Flavonoids and terpenoids are compounds which are present commonly in plants, have been reported to have a wide range of biological activities including antioxidant properties. The presence of terpenoids and flavonoids in this study were also confirmed by the qualitative analysis of *Salmalia insignis* and hence supported the therapeutic potentials of the plant drug under study.

Transverse section of stem of *Salmalia insignis* treated with Mayers reagents showed the presence of alkaloids (Brown colour) in the outer epidermal region and in vascular bundles (Plate 4 – a). It indicates the presence of alkaloids as well as synthesis, occurrence and distribution of tested secondary metabolites. Plate 4 – b shows the presence of tannin in the region of cortex when the sections were treated with potassium iodide solution. When the section were treated with 25% lead acetate solution showed the presence of flavonoids in the inner region of vascular supply (yellow colour) (Plate 4 – c).

For detection of starch the sections were treated with Lugol's iodine solution which shows positive result (Red colour) (Plate 4 – d). Sudan -1 reagent was used to detect total lipids. The dark stained region (Black colour) indicates the presence of lipids (Plate 4 – e). Plate 4 – f shows the presence of phenols in the region of cortex when

Table 5: Estimation of total phenolics and total flavonoid content of different solvent extracts of *Salmalia insignis* leaf powder.

S. No.	Extraction Medium	Total phenolics (mg TAE/g extract) [#]	Total flavonoid (mg RE/g extract) [#]
1.	Petroleum ether	54.8 ± 0.8	16.5 ± 1.0
2.	Ethanol	94.7 ± 3.6	73.1 ± 0.9

[#] Values are means of three independent analysis ± Standard Deviation

TAE - Tannic acid equivalent; RE - Rutin equivalent

the sections were treated with 5% ferric chloride anhydrous solution.

In the present investigation, we have reported the data obtained on histochemical studies on *Salmalia insignis* leaf and stem concluded the presence of polyphenols, flavonoids, tannins, starch and lignin which are of great medicinal value and finds extensive use in the drug and pharmaceutical industry. Results from this work therefore supports that the *Salmalia insignis* could be used as an alternative in the management of various ailments such as anticancer and wound healer.

The cell arrangements, size and shape of cells, shape and size of the crystals, starch grains, stomatal types and other specific micromorphological characters were studied using Photonic microscope (Model A X 70 TRF, Olympus optical) with a camera. The colour of the powder changed from Pale green to Yellowish Green. Diagnostic microscopic features of the powder include calcium oxalate prisms and clusters, starch grains and orange-colored matter (Plate 5).

Physico-chemical studies

Organoleptic characters of plant powder and the plant extract

The plant powder showed characteristic odour and bitter taste. Upon drying and powdering the colour of the powder changed from Pale green to Yellowish Green as shown in Table 1. The organoleptic characters such as colour, consistency and odour were noted in the petroleum ether and ethanolic leaf extracts of *Salmalia insignis* (Table 2).

Behaviour of plant powder with different chemical reagents

The behaviour of plant powder with various reagents were observed and presented in Table 3. Yellowish Green to Pale yellow was noted in the powder with different chemical reagents.

Qualitative phytochemical evaluation

The results of the preliminary phytochemical screening of the study plant showed the presence of various phytochemicals (Table 4). The petroleum ether extract revealed the presence of alkaloid, phenols, coumarin and gum and mucilage. Ethanolic extract concealed the presence of flavonoid, tannins, terpenoides, coumarin, quinone and fixed oil.

Total phenolics

Total phenolic content of different solvent extracts of *Salmalia insignis* were studied and expressed as tannic

acid equivalent. As shown in Table 5, the total phenolic content was maximum in ethanolic extract (94.7 ± 3.6 mg/g) followed by petroleum ether extract (56.8 ± 0.8 mg/g).

Total flavonoid content

Total flavonoid content of different solvent extracts of *Salmalia insignis* plant powder was studied and expressed as rutin equivalent and shown in Table 5. The total flavonoid content was maximum in ethanolic extract (73.1 ± 0.9 mg/g) followed by petroleum ether extracts (16.5 ± 1.0 mg/g).

Antioxidant activity

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of these diseases, antioxidant therapy has gained utmost importance.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was assessed by generating the hydroxyl radicals using ascorbic acid - iron EDTA. The hydroxyl radicals formed by the oxidation, reacts with dimethyl sulfoxide to yield formaldehyde, which provide a convenient method to detect hydroxyl radicals by treatment with Nash reagent. The hydroxyl radical scavenging activities of different solvent extracts of *Salmalia insignis* plant powder are presented in Table 6. In the present investigation, all the samples exhibited percentage activity between 0.097% and 26.78 %. All the extracts showed a dose dependent increase in hydroxyl radical scavenging activity. Among the extracts, the ethanolic extract appears to have the highest potential for hydroxyl radical scavenging activity. This value is comparable with the percentage value of the standard BHA (Fig. 1). In the present study, radical scavenging activity of the extract is moderate when compared to standard (BHA) (Plate - 3).

Statistical analysis

The antioxidant property of leaf powder extract of *Salmalia insignis* was indicated by colour changes of the solution. All experiments were performed in triplicates and the results are presented as mean ± SD (Standard Deviation).

DISCUSSION

Botanical identification of a phytodrug involves two steps. One is identification of the plant by its floral characters and the other is diagnosis of the plant with its microscopic characters. The latter procedure is useful for identification of fragmentary plant specimens. Certain microscopical characters are vulnerable for changes due to environmental stress. Yet, there are many anatomical features that are least modified by external factors and such features are specific at the species level or genus and family level. Early plant morphologist Robert Hook (1605 - 1703) clearly demonstrated that each kind of plant has its own distinctive structure by means of which it can be recognized.

For the utilization of medicinal plant as a biosource/ or to utilize a plant as a biosource or herbal drug, correct identification and quality assurance of the starting materials is an essential prerequisite. Therefore the

Table 6: Hydroxyl radical scavenging activity of different solvent extracts of *Salmalia insignis*.

S. No.	Sample	Concentration (µg/ml)	% activity #
		20	0.097 ± 0.02
		40	0.283 ± 0.005
	SIP	60	0.439 ± 0.02
		80	0.774 ± 0.04
		100	1.993 ± 0.01
		20	0.301 ± 0.03
		40	0.477 ± 0.014
	SIE	60	0.887 ± 0.03
		80	1.412 ± 0.024
		100	2.396 ± 0.013
		2	12.63 ± 0.7
		4	16.64 ± 0.6
	BHA	6	20.64 ± 1.0
		8	21.46 ± 2.3
		10	26.78 ± 1.4

Values are means of triplicate determinations ± Standard Deviation

SIP - Petroleum ether extract of *Salmalia insignis*

SIE - Ethanolic extract of *Salmalia insignis*

BHA - Butylated hydroxynisole

evaluation of a crude drug is an integral part for establishing the correct identification of a plant material to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Before including any crude drug in herbal pharmacopoeia, its pharmacognostic parameters and standards must be established (Kumar *et al.*, 2010).

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as alkaloids, flavonoids, phenolics, cardiac sterols, triterpenoids, saponins and carbohydrates. Such a study would serve as a useful tool in standardization of the leaf material, isolation of medicinally important phytoconstituents, performing pharmacological investigations and ensuring quality formulations in the future. It would also help in distinguishing the plant material of *Bombax insigne* from its related species, *Bombax ceiba* (Pandya *et al.*, 2010).

The present macroscopical investigations of *Salmalia insignis* revealed that the species is large deciduous tree, reaching 30 to 40 meters in height and up to three meters trunk diameter (Plate-1). The fresh leaf is green in color with characteristic odour and slight bitter taste. The leaves are compound, pinnately trifoliate with 7-9 leaflets arranged on a common petiole, 7 to 10 inches long with entire margins and deciduous, being shed in the dry-season. Several earlier workers have adopted macroscopical features as one of the effective parameters for the pharmacognostical identification of several plant derived crude drugs (Shantha *et al.*, 1985; Rivera-Arce *et al.*, 2007; Pandya *et al.*, 2010).

The present anatomical study provides a set of characters specific for *Salmalia insignis* with which one can establish

the identity of the plant in fragmentary form. Transverse section of the leaf shows a thick midrib, lateral veins and thin dorsiventral lamina having a single layer of compact rectangular epidermal cells with a thick cuticle. Beneath it is a single, compact layer of radially elongated palisade cells followed by loosely arranged spongy mesophyll cells rich in starch grains. Mid-rib consists of well-developed collenchymas cells, present just below the upper epidermis and above the lower epidermis. A sheath of calcium oxalate cluster crystals is present above the lower epidermis in the lower collenchyma of the mid-rib. Ground tissue consists of loosely arranged polygonal parenchymatous cells having orange coloring matter. Vascular bundles are bicollateral. Primary vascular bundles showed 14 - 15 seriate xylem vessels whereas secondary vascular bundle showed 4 - 5 seriate xylem vessels. Bunch of pericyclicfibres are situated above the secondary vascular bundles, whereas they are discontinuous below the primary vascular bundles (Plate 2). Anomocytic stomata and no specific subsidiary cells are additional diagnostic features (Plate 5). This is in corroboration with the work of Pandya *et al.*, (2010) and Khin Maung Sint *et al.* (2013).

Khin Maung Sint *et al.* (2013) reported the wood anatomical characteristics, content of phenolic extractives and topochemistry of *Bombax ceiba* and *Bombax insigne* showed some light differences in the quantitative wood anatomical data among the regions due to the influence of environmental conditions. The amount of phenolic extractives obtained by gradual extraction with acetone-water was almost the same in heartwood and sapwood (about 1.2%) in *B. insigne*, while heartwood showed a higher amount (2.8%) than sapwood (2.5%) in *B. ceiba*. This is in agreement with the present observation. Stem cortex in *Salmalia insignis* (Plate 3) is wider with vascular cylinders of several wide wedge shaped xylem segments. The xylem segments have thin walled vessels and thick blocks of phloem. Secondary growth appeared in old stem where the xylem cylinder is thick with rows of continuous primary xylem. Secondary xylem includes vessels and lignified fibres.

Microscopic examination of leaf powder is one of the methods adapted to find the adulteration of the powder form. To detect any adulteration one must have the knowledge of the powder characteristics of the original drug and if possible the knowledge of those materials that are usually employed for adulteration. A leaf powder may exhibit fragments of epidermal cells with or without stomata, cell inclusions such as starch grains and crystals (Plate 5). This is in accordance with the result of Pragasam (2010).

Starch is the principal ergastic substance of the protoplast. It is composed of long chain molecules, whose basic units are anhydrous glucose residues. In starch granules the molecule is radially arranged, therefore, in polarized light a cross pattern is seen. The morph metric variation of starch grain is so extensive that they may be used taxonomically and pharmacognostically up to a limited extent (Kuster and Die pflanzenzelle, 1956).

Tannin is a heterogeneous group of phenol derivatives, usually related to glucosides. Tannins are particularly abundant in the leaves of much plant; in the xylem, in the testa of seeds and in pathological growth like galls. Fats are widely distributed in the plant body and they probably occur in small amounts in every plant cell.

Sensory or organoleptic characters describe colour, odour, taste and fracture of the raw materials used for drug preparation, identification and adulteration. Present organoleptic investigation of leaf powder of *Salmalia insignis* exhibited characteristic smell and bitter taste (Table 2). Organoleptic profile is one of the many diagnostic parameters in the proper identification of raw materials (Shanmugavadivu and Subramanian, 2009).

The preliminary phytochemical investigation of the present study provides correlative data for the diagnosis, purity and quality of the drug. The results of the preliminary phytochemical screening of the study plant showed the presence of various phytochemicals (Table 5). The petroleum ether extract revealed the presence of alkaloid, phenols, coumarin and gum and mucilage. Ethanolic extract concealed the presence of flavonoid, tannins, terpenoides, coumarin, quinone and fixed oil. Glycosides, saponin, anthroquinone, carbohydrate, proteins and amino acids are totally absent in all the two extracts. The present findings coincide with their results of Pandya et al., (2010).

CONCLUSION

The present work deals with the microscopic, physicochemical and phytochemical evaluation of the leaves of *Salmalia insignis*. Main microscopic characters include anomocytic stomata, sheath of calcium oxalate cluster crystals present above the lower epidermis of the mid-rib and pericyclic fibres in groups above the secondary vascular bundles and discontinuous below the primary vascular bundles. Diagnostic characters of powder include bundle of pericyclic fibres, calcium oxalate clusters and prisms, starch grains and xylem vessels with reticulate or annular thickening.

Various physicochemical parameters were established which can be important in detecting adulteration and mishandling of the crude drug. Phytochemical analysis showed the presence of many important classes of phytoconstituents like alkaloids, flavonoids, phenolics, cardiac sterols, triterpenoids, saponins and carbohydrates, which may influence the pharmacological actions of the plant. Such a detailed study would be decisive in performing standardization of the leaf material, preparation of its monograph, isolation of phytoconstituents, performing further pre-clinical and clinical investigations and manufacturing of its formulations and differentiating it from its closely related species, *Bombax ceiba*. It is assumed that macroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almost entirely on macroscopical characters. The results further improve the knowledge on the wood anatomy and chemistry of the species and in this respect are useful in future research to

broaden their utilization potential. These anatomical characters can be used to identify medicinal plants and may be applicable to the quality control.

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