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Pharmacognostic and Phytochemical Investigation of the Aerial Parts of *Walsura trifoliata* (A. Juss.) Harms

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ABSTRACT

Objective: The present study deals with the pharmacognostical examination include microscopical characters and phytochemical studies of aerial parts of *Walsura trifoliata* which is known as 'Kanjimaram', belongs to the family Meliaceae. The plant is well reputed in traditional system of medicine. The present study furnishes a comprehensive data for the anatomical features of the leaf, lamina, petiole and stem of *W. trifoliata*. **Methods:** Anatomical studies of leaf and stem carried out by employing the customary techniques of microtomy and photomicrography. Fluorescence characters, ash value and extractive values of aerial parts of *W .trifoliata* have been determined by the methods of Chase and Pratt, 1949. Qualitative organic analysis of aerial parts of the plant in alcoholic extracts has been performed. **Results:** Physicochemical parameters of *W. trifoliata* showed loss on drying 7.01% and total ash 4.97%. The weight of the ash left behind after the combustion is of important parameter for the standardization of drug. Microscopic characters of different parts such as leaf, petiole and stem constitute the reliable features for botanical identity of the plant. **Conclusion:** The present study provided useful information about its correct identity and evaluation. It helps to differentiate from the closely related species of *Walsura*.

INTRODUCTION

Walsura trifoliata (A. Juss.) Harms. belongs to the family Meliaceae (Syn: *Walsura piscidia* Roxb., *Heynea trifoliata* A. Juss). It is an evergreen tree distributed widely in the tropical areas of Asia, such as Southern China, India, Malaysia, and Indonesia[5]. It grows on dry deciduous forests of 200 to 300 m height. This plant is well reputed in traditional system of medicine and used by tribal peoples to treat various diseases like skin allergies, astringent and diarrhoea[9]. The bark of the plant is reported to posses stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison[1]. The bark extract of *Walsura trifoliata* showed the activity against pathogenic microorganisms[10]. Bhadane and Patil studied leaf epidermal features of Walsura *trifoliata* [3]. A lacuna in the pharmacopoeia of the plant prompted the present investigation.

MATERIALS AND METHODS

Walsura trifoliata was collected from Western Ghats and identified by Dr.V. Chelladurai, Rtd Senior Research Officer, Tirunelveli, with the help of the Flora of presidency of Madras[7] and Flora of Tirunelveli hills (Southern Western Ghats)[8]. Voucher specimens have been deposited at St. John's College, PG and Research department of Botany, Palayamkottai, Tamilnadu (Voucher No. SJCH 936).

Anatomical studies of leaf and stem carried out by employing the customary techniques of microtomy and photomicrography. The materials were macerated using Jeffrey's solution to study the cellular components. Fluorescent analysis of the aerial parts powder in different solvents were carried out according to the methods of Chase and Pratt [4]. Physicochemical characters were determined by standard methods[2].

RESULTS

Macroscopic features

Trees upto 15 m tall, branchlets tawny-pubescent, leaves compound, trifoliate, alternate, glabrous; lamina 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 gradually curved, tertiary nerves broadly reticulate, slender (Fig 1).

Inflorescence terminal or axillary panicles; flowers pentamerous, bisexual, greenish yellow in colour. Fruit is an ovoid berry; seeds enclosed in a white fleshy aril.

Microscopic features

Leaf

The leaf in trans sectional view exhibits smooth adaxial surface and prominent midrib with adaxial concavity (Figure 2.1). The midrib is concavo-convex having wide shallow adaxial concavity and thick convex on the abaxial side. The midrib 500 µm in vertical section and 500 µm in horizontal plane; it is 450 µm in vertical plane (Figure 2.2). 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 (Figure 2.2).

Figure 1. A flowering twig of *Walsura trifoliata*

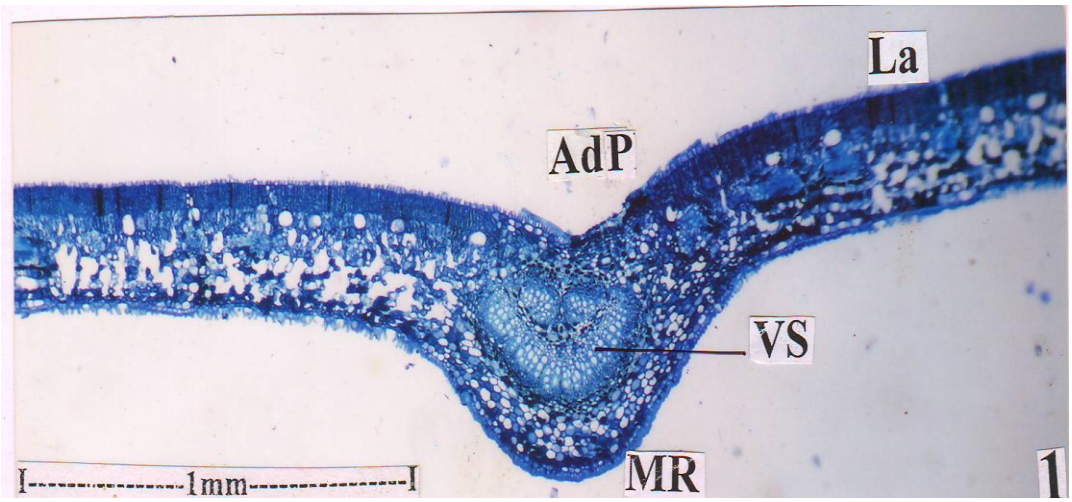


Figure 2.1. T. S. of leaf through midrib

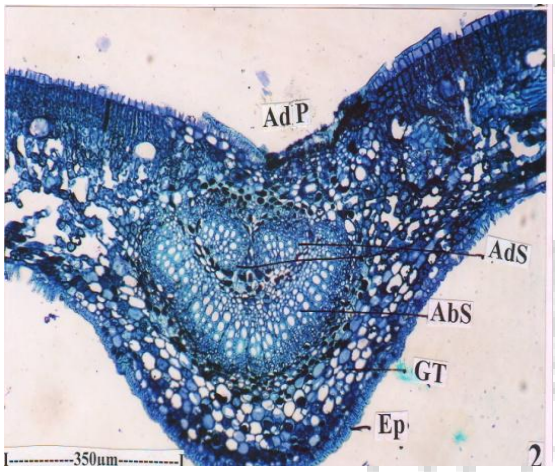


Figure 2.2. T. S. of midrib enlarged

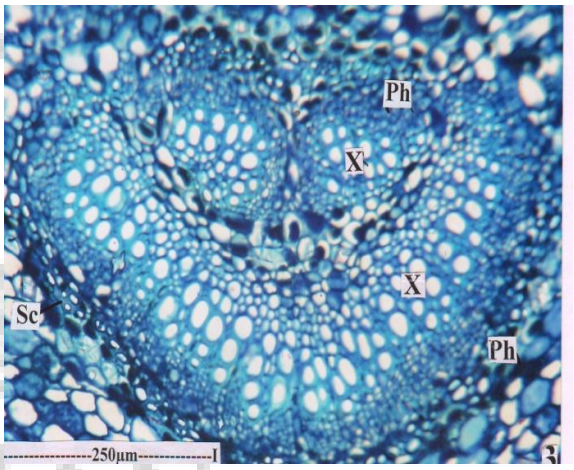


Figure 2.3. Vascular strands of the midrib enlarged

Figure 2.1, 2.2 & 2.3 AbS- Abaxial strand, AdP– Adaxial part, AdS– Adaxial strand, Ep– Epidermis, GT– Ground tissue, La- Lamina, MR– Midrib, Ph– Phloem, VS– Vascular strand, X– Xylem.

The vascular system includes two, circular masses of collateral strands located in the adaxial side and one, wide, bowl shaped abaxial strand (Figure 2.2 & 2.3). 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 (Figure 2.3).

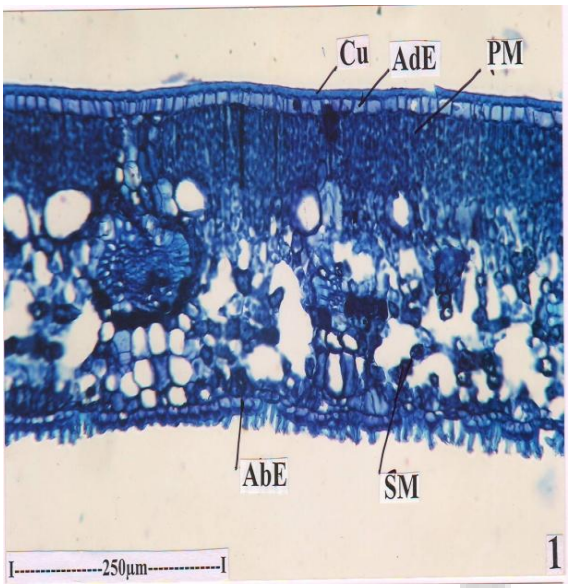


Figure 3.1. T.S. of Lamina

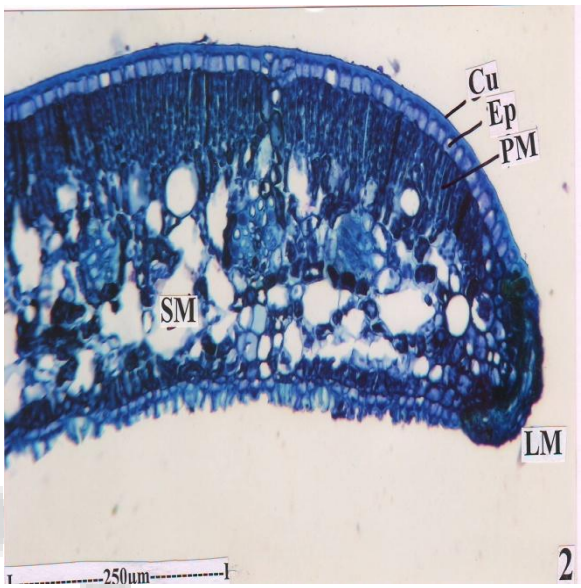


Figure 3.2. T.S. of leaf margin

Figure 3.1 & 3.2 AbE– Abaxial epidermis, AdE– Adaxial epidermis, Cu– Cuticle, Ep– Epidermis, LM– Leaf margin, PM– Palisade mesophyll, SM– Spongy mesophyll.

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 260 µm thick (Figure 3.1). 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 200 µm 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 (Figure3.2).

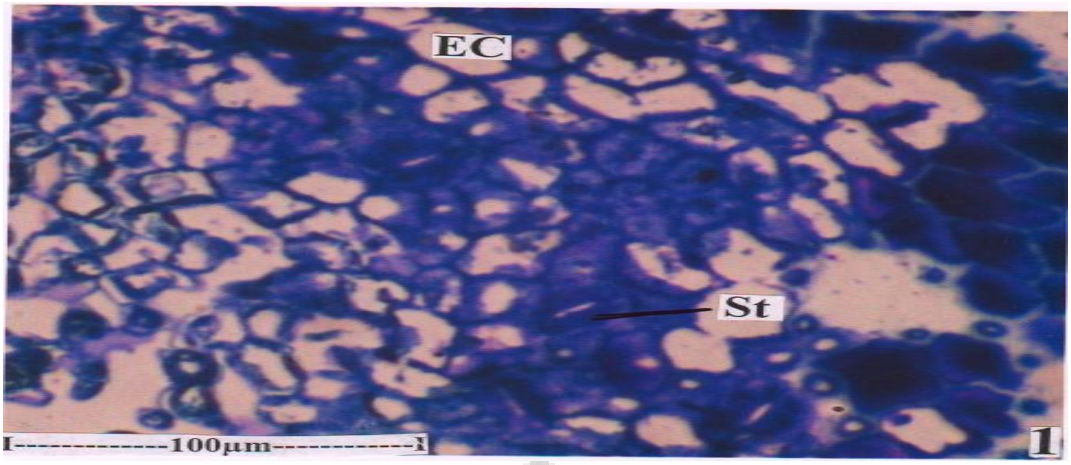
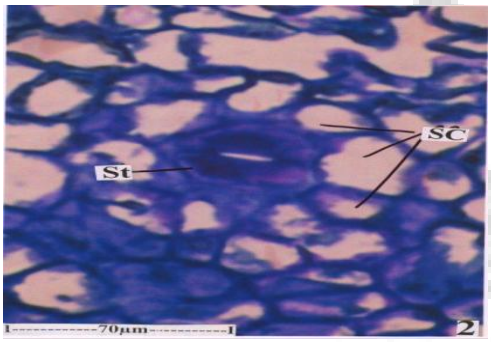


Figure 4.1. Paradermal section of the lamina showing the stomata



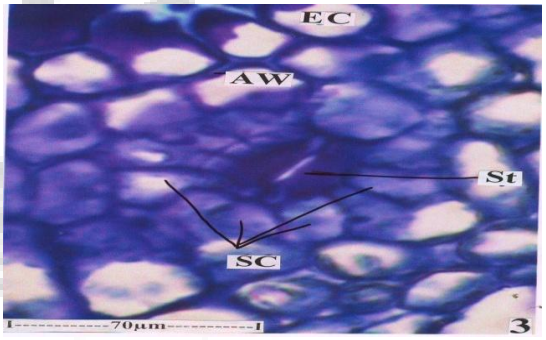


Figure 4.2. & 4.3. Cyclocytic stomata enlarged

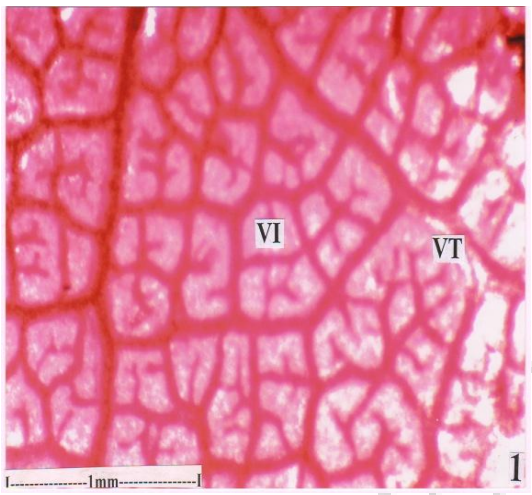
Figure 4.1, 4.2 & 4.3 AW– Aniclinal wall, Ec– Epidermal cell, SC– Subsidiary cell, St– Stomata.

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 stomata appears to be stellate stomata (Figure 4.1, 4.2 & 4.3). The guard cells are broadly elliptical measuring 20 × 20 µm in size. The stomatal aperture is narrow and slit like (Figure 4.2 & 4.3).

Venation pattern of the lamina

The veins and veinlets are thick and straight. They form fairly wide vein islets with well defined thick and straight vein boundaries (Figure 5.1). 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, thick and curved (Figure 5.2).



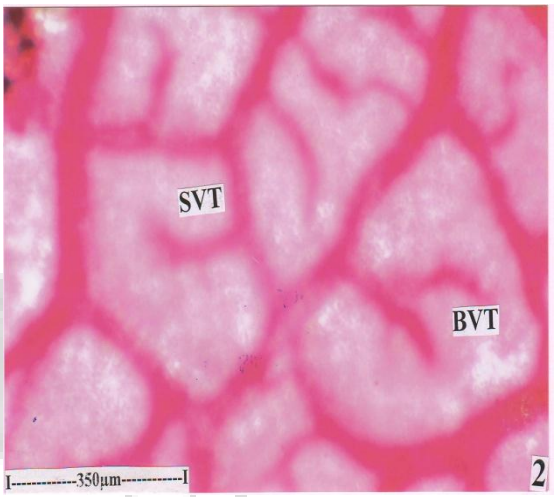


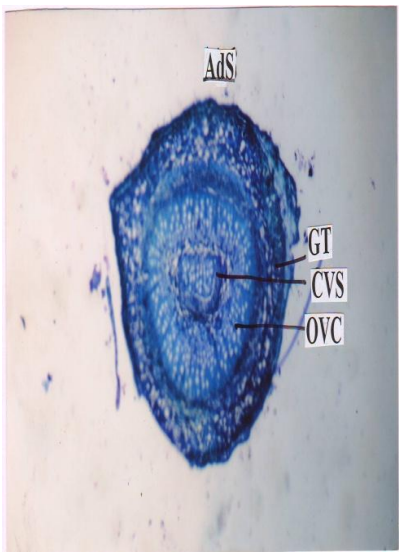
Figure 5.1. Venation pattern of the lamina **Figure 5.2. Vein islet and vein terminations enlarged**

Figure 5.1 & 5.2 BVT– Branched vein termination, SVT– Simple vein termination, VI– Vein islet, VT– Vein termination.

Petiole

The petiole is circular with short 2 lateral wings. It is 1 mm thick. The petiole consists of thin epidermal layer which is often broken due to growth in diameter of the petiole (Figure 6.1). 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 (Figure 6.2).



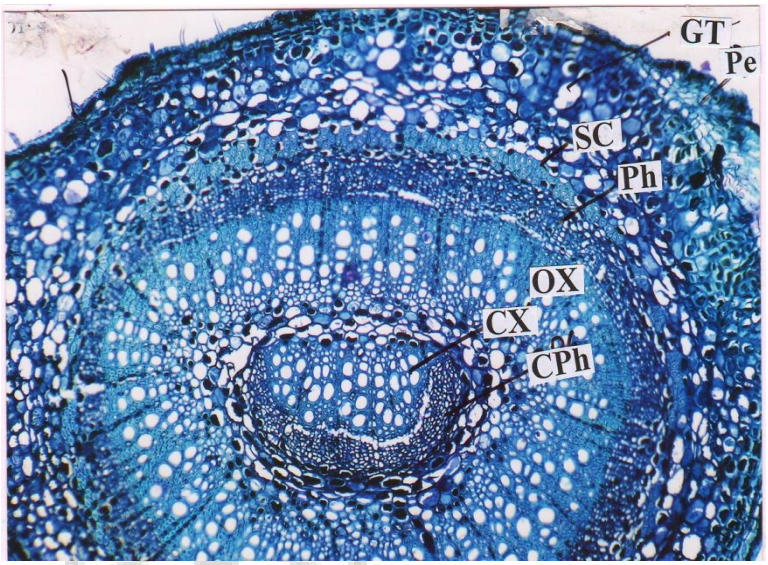


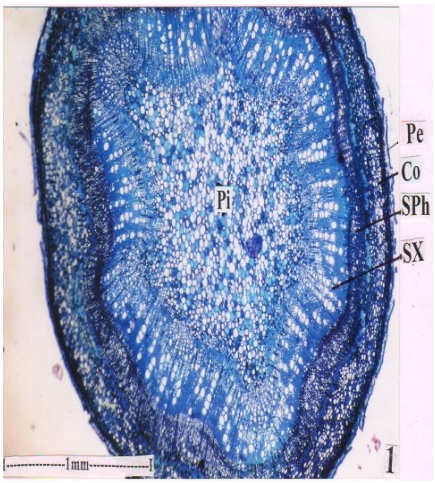
Figure 6.1. T.S. of Petiole entire view **Figure 6.2. T. S. of Petiole a sector enlarged**

Figure 6.1 & 6.2 AS– Adaxial side, CVS– Central Vascular Strand, CX– Central Xylem, GT– Ground tissue, OX– Outer Xylem, OVC– Outer Vascular Cylinder, Ph– Phloem, SC– Sclerenchyma, Pe– Periderm.

Stem

The stem is circular in outline with epidermis, cortex, sclerenchyma cylinder, secondary phloem and secondary xylem. The stem is 2.35 mm thick (Figure 7.1). 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 50 µm in thickness. The boundary layer of the cortex is marked by a few isolated irregular masses of sclerenchymatous cells (Figure 7.2). The secondary phloem is quite thick comprising 2 or 3 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 they are thin and straight running from secondary xylem to secondary phloem. The vessels are circular and the secondary xylem vessels are upto 30 µm wide. The pith includes both thin walled parenchyma and thick walled fibres. These two types of cells are mixed with each other (Figure 8).



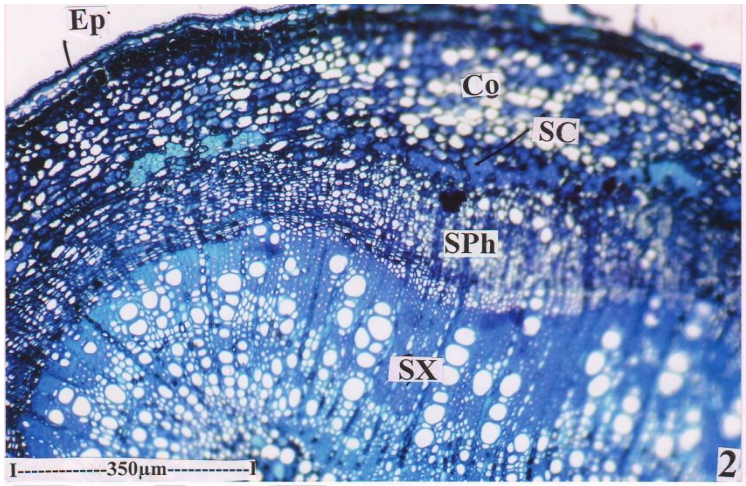


Figure 7.1. T.S. of stem entire view **Figure 7.2. T.S. of stem a sector enlarged**

Figure 7.1 & 7.2 Co– Cortex, Ep– Epidermis, Pe– Periderm, Pi– Pith, Sc– Sclerenchyma, SPh– Secondary Phloem, SX– Secondary Xylem.

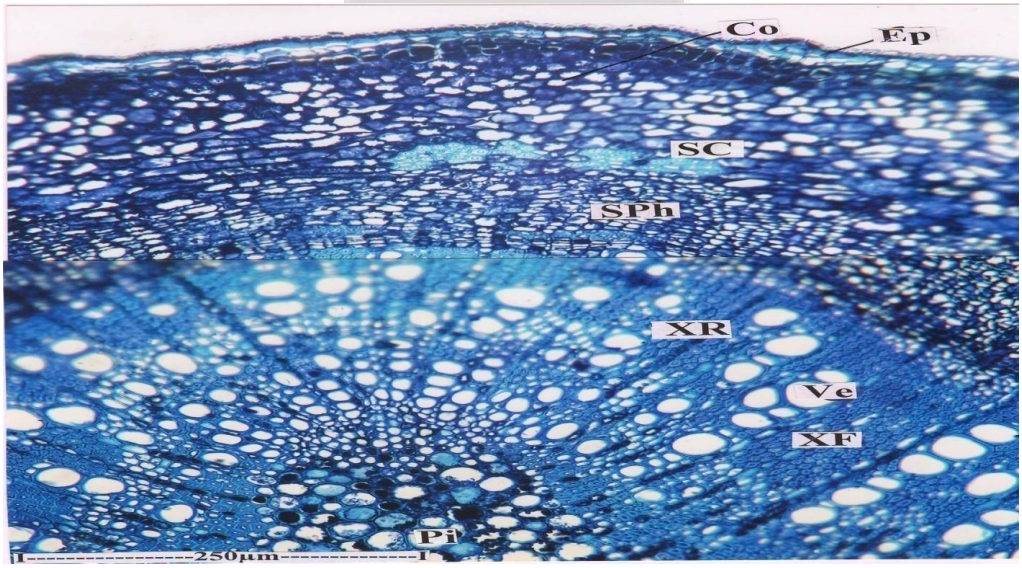


Figure 8. T.S. of stem showing cortical zone, secondary phloem and secondary xylem

Figure 8. Co– Cortex, Ep– Epidermis, SC– Sclerenchyma, SPh– Secondary Phloem, Ve– Vessel, XF– Xylem fibre, XR– Xylem ray.

Preliminary Phytochemical Screening

The preliminary phytochemical screening with the various qualitative chemical tests and fluorescence analysis were carried out. The results were shown in Table 1, 2 and 3.

Table 1: Determination of physical constants

Sr. No.	Parameters	Results (% w/w)
1.	Total Ash	4.97
2.	Water soluble ash	1.17
3.	Acid insoluble ash	0.34
4.	Loss on drying at 105^0 c	7.01
5.	Water soluble extractive	15.10
6.	Alcohol soluble extractive	10.09
7.	Extractive value (Successive extraction)	
	Hexane	1.3
	Petroleum ether	2.52
	Chloroform	2.73
	Ethyl alcohol	4.18

Table 2: Preliminary phytochemical screening of ethanolic extract of *Walsura trifoliata* (aerialparts)

Steroid	Negative
Triterpenoid	Positive
Flavonoid	Positive
Furan	Positive
Sugar	Positive
Coumarin	Positive
Quinone	Positive
Alkaloid	Negative
Tannin	Positive
Phenol	Positive
Acid	Negative
Saponin	Negative

Table.3 Fluorescence analysis of the aerial parts of *Walsura trifoliata*

Sr. No.	Treatment	Under visible light	Under UV 265 nm	Under UV 365 nm
1.	Powder	Pale green	Palegreen	Reddish brown
2.	Powder+Petroleum ether	Pale green	Dark green	Dark brown
3.	Powder+Hexane	Pale green	Pale green	Blackish brown
4.	Powder+Chloroform	Dark green	Brown	Black
5.	Powder+Ethylacetate	Pale green	Dark green	Black
6.	Powder+Ethyl alcohol	Orange	Light green	Black
7.	Powder+Distilled water	Light brown	Pale green	Brown
8.	Powder+1N NaOH	Reddish brown	Dark green	Blackish brown
9.	Powder+1N HCL	Light brown	Pale green	Dark brown

DISCUSSION

Standardization is an important tool in identifying crude drug correctly. For establishing the correct identity of source materials, microscopic method is one of the simplest and best methods. Therefore, the results of present study, may serve as a basis for identification, collection and standardization of the plant.

Microscopical study of leaf showed the presence of finger like trichome, accumulation of tannin, formation of wide air chambers, sunken and cyclocytic stomata. The veins and veinlets are thick and straight. The petiole is circular with short two lateral wings. The phloem elements occur in the form of wide hollow cup on the lower arc of the xylem strand. The stem showed the presence of spiny cuticle and irregular mass of sclerenchymatous cells in the cortex. These characters constitute the reliable features for botanical identity of the plant.

Physicochemical parameters of *W. trifoliata* showed loss on drying 7.01 % and total ash 4.97 %, the weight of the ash left behind after the combustion is of important parameter for the standardization of drug. Every part of a plant provides a particular amount of ash. The weight of total ash therefore gives information whether it is adultered with any other organic or inorganic materials. *W. trifoliata* contains the acid insoluble ash 0.34 % and water soluble ash 2.17 %. The

acid insoluble ash gives an idea about the earthy matter and other impurities which might be present along with drug.

Extractive values of the plant with different solvents give a preliminary picture of the percentage of the compounds extracted. In *W. trifoliata* maximum extractive value was found with ethanol (3.6 %) minimum (0.96 %) with n-hexane.

This result shows the solvent ethanol is preferable to other solvent for the yield of more of the compounds.

The extract of *W. trifoliata* is exposed to UV light, it exhibits fluorescent effects, that provides evidence for the presence of fluourescent compounds.

Qualitative tests carried on the aerial parts of *W trifoliata* confirmed the presence of various pharmacologically important plant constituents like triterpenoid, phenol, flavonoid, coumarin, quinine, furan and glucoside/sugars. Alkaloids, steroids and saponins are absent in the extract. For instance, the presence of tannins may be responsible for ability of *W. trifoliata* to cure diseases such as diabetes, diarrhea, sore throat, skin ulcer and dysentery. The presence of flavonoids in *W. trifoliata* may be responsible for its uses to cure cancer, inflammations and allergies[6].

CONCLUSION

The present study provided useful information about its correct identity and evaluation. It helps to differentiate from the closely related species of *Walsura*. This is also useful for the future identification of the plant, and serves as a standard monograph for identification and evaluation of plant.

ACKNOWLEDGEMENT

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