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Original Article

CYTOMORPHOLOGICAL AND PHYSICO-CHEMICAL STUDIES OF *SIDA RHOMBIFOLIA*

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ABSTRACT

Objective: To investigate the cytomorphological and physico-chemical characteristics of *Sida rhombifolia*.

Methods: Fresh leaves, stems and roots were studied for microscopical characters and dried powder of leaves, stems and roots were subjected to physico-chemical analysis using standard methods.

Results: The detail microscopy of leaf revealed the presence of paracytic stomata, unicellular covering trichome, cluster of calcium oxalate crystals, lignified xylem, non-lignified phloem; presence of both simple and compound starch granules, pitted, spiral and annular xylem vessels were found in stem; and section of root found to contain cortex of polygonal and rectangular cells with brownish content, both lignified and nonlignified fibres, xylem vessels of spiral, annular thickening, and squarish type of crystals. Leaf constants such as stomatal number, stomatal index, and vein-islet number along with the dimension of starch grains were measured. Physico-chemical parameters such as ash values, extractive values, loss on drying, fluorescence analysis, behavior of powder drug with different chemical reagents, etc. were also determined. Preliminary photochemical screening of plant parts showed the presence of alkaloids, glycosides, flavonoids, phenolics, tannins, saponins, steroids and amino acids.

Conclusion: The microscopic study and physico-chemical analysis of *Sida rhombifolia* (*S. rhombifolia)* are useful in standardization for detrmination of quality, checking of purity and identification of the sample.

Keywords: *Sida rhombifolia*, Microscopic study, Physico-chemical investigations, Fluorescence analysis.

INTRODUCTION

Nowadays, herbal drugs play an important role for the treatment of various diseases especially in developing countries. Plants provide various novel bioactive compounds with ethnobotanical informations since many species are being used in traditional systems of medicine. The demand of herbal drugs has been increased remarkably and the global trend is people are returning to the natural therapies [1]. The lack of quality control measure and proper documentation of alternative medicines are the major drawbacks for the acceptance of alternative medicines in developed countries. The documentation and standardization of the raw materials used in herbal medicine is very essential for global acceptance of these traditional systems of medicines [2]. Microscopical and physico-chemical evaluation studies were globally accepted for authentication of the medicinal plant materials. The correct botanical identification and physico-chemical study of raw plant materials are vitally necessary to ensure reproducible quality of herbal medicines for its safety and efficacy. The plants grow in various geographical locations with diverse vernacular names show great fluctuations in their quality. The quality control of medicinal plants plays an important role to exchange relevant informations among the scientific research community [3]. Thus, standardization of medicinal plants has been extensively taken into consideration during the recent years. One such plant, *Sida rhombifolia* is undertaken for standardization as it has not been previously studied for its microscopical and physico-chemical characters.

Sida rhombifolia Linn. (*S. rhombifolia*) belongs to the family Malvaceae, which is commonly known as Barela and Paddy's lucerne. It is called as Atibala, Pitapuspi, Barela etc. in Sanskrit, and Pitabala, Sahadebi, Pitabariyar etc. in Hindi. It is a small erect woody, annual or perennial under shrub of about 1.5 m high with rough branches and stellate hairs. Leaves are very variable in shape up to 5 mm by 18 mm, short petioled, rhomboid-lanceolate to lanceolate, serrated towards the top and entire towards the base. The flowering and fruiting in the plant starts from September to December. Flowers are yellow or white, solitary or in pairs [4].

In India, the decoction of entire plant of *S. rhombifolia* reduces rheumatic pain when given orally to human adults. For the same purpose, the decoction mixed with equal proportion of cow's milk is also taken every morning for about a week [5]. The fresh plant juice in India, is taken orally to dissolve stones in the urinary tract while in Nepal; the plant juice is applied externally for boils [6, 7]. In India, the decoction of the root is used orally for treatment of pulmonary tuberculosis and the aqueous root extract is used to treat malaria [8, 9].

In Unani system of medicine, the leaves and roots are used in case of piles and gonorrhoea. The roots and leaves are sweetish, aphrodisiac, tonic, remove tridosha and good in urinary complaints such as discharges and strangury. These are also useful in fever, heart diseases, burning sensations and all kinds of inflammation. The root is used in the treatment of rheumatism. In Europe, the plant has been regarded as the valuable remedy in pulmonary tuberculosis and rheumatism. In Madagascar, the plant is mostly used as an emollient; an infusion of the root is given in dysentery; the leaves are pounded and applied to tumours or chewed and applied to boils. It is also useful in the antidotal treatment of snake-bite and scorpion-sting [10]. The crude extract of root (5 g/kg and 10 g/kg) found to produce sedative effect and significant potentiation of pentobarbitone sleeping time in mice [11]. The infusion of dried leaf of *S. rhombifolia* in central Africa is used for diabetes, chest pain and diarrhoea on oral administration. The infusion of this plant is applied locally for the treatment of skin diseases and infected wounds [12]. Although microscopical and physico-chemical studies of this plant were previously published, the detailed cytomorphological studies along with physico-chemical studies of different parts of *S. rhombifolia* has not been described till date. Keeping all these into account, the detailed cytomorphological and physico-chemical studies of this plant are described in this research article for proper identification from its allied species.

MATERIALS AND METHODS

Collection and preparation of plant specimen

The plant specimen for the proposed study was collected from Bargarh, Odisha. The required sample of different organs was cut and removed from the plant and fixed on FAA (Formalin-5 ml+Acetic acid 5 ml+70 % Ethyl alcohol 90 ml). After 24 h of fixing, the

specimen was dehydrated with graded series of tertiary-butyl alcohol as per the reported method [13]. Infiltration of the specimen was carried out by gradual addition of paraffin wax (melting point 58 ˚C to 60 ˚C) until solution attained super saturation. Then the specimen was cast into paraffin wax blocks.

Sectioning and staining

The paraffin embedded specimen was sectioned with the help of Rotary Micrometer. The thickness of the specimen was maintained at 10-12 µm [14]**.** The section was stained with safranin and fast green. The dye renders deep red colour to lignin and bright green colour to cellulose [15]. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal section as well as clearing of leaf was done with 5 % sodium hydroxide or epidermal peeling by maceration employing Jeffrey's maceration fluid [13].

Photomicrographs

Photographs of different magnifications were taken with Nikon Lab photo 2 microscopic unit. For normal observation, bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed as these structures have birefringent property under polarized light in which they appear bright against dark background. Descriptive term of the anatomical features is used as given in the standard anatomy books [16].

Quantitative determination was carried out for measurement of diameter of starch grain and length of phloem fibre [17]. The physico-chemical studies [18], behavioral study of powder drug towards different chemical reagents [19] and fluorescence analysis [20] of various plant materials were also carried out.

Preliminary phytochemical screening of the plant extracts

Preliminary phytochemical screening was carried out using the standard methods [21].

RESULTS AND DISCUSSION

Macroscopic study

Leaves are ovate to oblong, often more or less rhomboid and 0.5-8 cm X 0.3-5 cm in size. Leaf base is mostly acute, sometimes rounded, truncate or slightly cordate, serrate to crenate in the upper part, entire towards the base, apex is acute to acuminate or obtuse and rarely rounded. Petiole is 0.2-1.5 cm long and stipules are filiform. Flowers are axillary, solitary or in clusters of 2-5. Pedicel is longer than the petiole, and 3.5 cm long. Calyx is campanulate, free upto or above the middle, 4-6 mm long and accrescent; lobes are triangular to ovate, 4-6 X 3-5 mm and acuminate. Corolla is yellow, 1-1.8 cm across; petals oblique, cuneate at base. Staminal column is shorter than corolla, hairy and glabrous. Mericarps are 7-12, occasionally with 2 awns, 1-2.5 cm long. Seeds are flattened, reniform and glabrous.

Quantitative microscopy

Quantitative microscopy is an important tool in the evaluation of crude drug. It is shown in fig. 17 (a-f). The leaf constants of *Sida rhombifolia* are presented in table 1. Epidermal cells are wavy. It contains anisocytic or cruciferous type of stomata, stellate (star shaped) multicellular unbranched trichomes, thick walled unicellular covering trichomes with bulbous base and tapering apex, and circartrix (scars/remains of trichomes).

Microscopic study

T. S of midrib of leaf

Microscopial characters of mid rib of the leaf are shown in fig. 1(a), fig.2 (a, b) and fig. 3 (a, b, c) at magnifications of 5 x×5 x, 5 x×10 x and 5 x×45 x respectively. Epidermis is single layer, composed of straight walled rectangular cells. Below the epidermis, 6-7 layers of collenchymatous cell are present. Collenchyma is followed by thin layer of parenchymatous cell of about 4-5 layers. 3-4 layers of collenchymatos cells are present above the lower epidermis. Vascular bundle is arc shaped. It consists of lignified xylem and non lignified phloem. Here, vascular bundle is closed due to absence of cambium. Vascular bundle is collateral closed (xylem and phloem are present on the same radius in the same vascular bundle). Xylem is endarch, where protoxylem lies towards centre and metaxylem towards periphery. Xylem is surrounded by phloem. Phloem contains clusture of calcium oxalate crystals. Phloem tissue is surrounded by pericycle fibre. It consists of multilayered sclerenchymatous cell which forms ring or patches just above the vascular bundle. These are highly lignified. The vascular bundle is completely surrounded by 3-4 layers above and 6-7 layers below the parenchymatous cell. Lower epidermis consists of single layer of polygonal parenchymatous cell and it contains covering trichomes on it surface.

T. S of lamina

The microscopical features found in the lamina of the plant are shown in fig. 3 (a, b) and fig. 3 (c, d) at magnifications of 5 x×10 x and 10 x×45 x respectively. Lamina of leaf is dorsiventral. Upper epidermis consists of single layer of rectangular parenchymatous cells. It shows the presence of unicellular covering trichomes and opening of stomata. Upper epidemis is followed by mesophyl. Mesophyl consists of palisade cell and spongy mesophyl. Palisade cells are arranged loosely in single layer, and are radially elongated. Spongy mesophyll contains 2-3 layers of loosely arranged rectangular parenchymatous cell. Mesophyl region is followed by lower epidermis. Lower epidermis is resembled to upper epidermis. Leaf contains stellate (star shaped) multicellular covering trichomes, which are considered as its characteristic feature.

T. S of petiole

Transverse sections of petiole are shown in fig. 4 (a), fig. 5(a), fig. 6 (a, b) and Fig.7(a, b) at magnification of 5 x×5 x, 5 x×10 x, 10 x×10 x and 5 x×45 x respectively. The epidermis consists of single layer of polygonal parenchymatous cell followed by 3-4 layers of collenchymatous cell. About 6-7 layers of parechymatous cells are present below the collenchymatous cell. Pericycle fibres are present as crown above the phloem part. It consists of thick walled sclerenchymatous cell which are lignified. Vascular bundle consists of xylem and phloem. It is closed due to absence of cambium. Xylem is endarch. Proto xylem is towards the center and metaxylem towards periphery makes the growth centrifugal. Xylem is surrounded by 6-7 layers of phloem on both sides. Phloem consists of cluster of calcium oxalate crystals. Vascular bundle is surrounded by ground tissue. Ground tissue consists of 3-4 layers of parenchymatous cell having entracellular spaces. At the center, pith is present, which consists of parenchymatous cell.

T. S of stem

Transverse sections of stem are shown in fig. 8(a), fig. 9(a,b) and Fig.10(a,b, c) at magnification of 5 x×5 x, 5 x×10 x and 5 x×45 x respectively. The epidermis consists of single layer, rectangular shaped, closely packed parenchymatous cell without having intercellular spaces. Cork cell consists of 2-3 layers of parenchymatous cell present below the epidermis. Cork cell is followed by phellogen, which are about two layers of rectangular parenchymatous cell. Below the phellogen, cortex region is present, which is about 8-9 layers of parenchymatous cell. In the cortex zone, groups of pericycle fibres are present.

It is about 3-9 in numbers in each group. It consists of thick walled sclerenchymatous cell which are highly lignified. Cortex region is followed by vascular bundle, consisting of xylem and phloem. Arrangement of the vascular bundle is collateral. Phloem consists of isodiametric parenchymatous cells which are about 9-10 layers, few cells contain cluster of calcium oxalate crystal. Xylem vessels are lignified and it may have reticulate, annular or spiral thickening. Xylem vessels are surrounded by lignified xylem parenchyma. Medullary rays are present which are biseriate or multiseriate. It becomes diverge at phloem region and converge towards pith region. Pith occupies the major part at the center. It consists of parenchymatous cell with intercellular spaces. At the center, parenchymatous cells are bigger in size and it becomes smaller towards periphery. Few cells of pith contain cluster of calcium oxalate crystals.

T. S of root

Various microscopical characters of the root are presented in fig. 11(a), fig. 12(a, b) and fig. 13(a, b, c). Cork consists of 3-4 layers of thin-walled flattened rectangular cells with reddish brown content. Cork region is followed by cortex, which is consisting of 17-18 layers of thin-walled tangentially elongated parenchymatous cell. In the cortex region 7-8 patches of pericycle fibres, made up of lignified sclerenchymatous cell, are observed. These are present above the vascular bundle. Vascular bundles are consisting of xylem and phloem. These are closed due to absence of cambium and are arranged in collateral. Patches of phloem are present on the outer side of xylem. Phloem tissues are present in 6-7 layers above xylem. Xylem occupies the central part of the root. Xylem vessels are big either single or in groups of few. The vessels are lignified, reticulate, and annular or spiral. Xylem vessels are surrounded by xylem parenchymas which are lignified. Medullary rays are present between the xylem vessels and xylem parenchyma. These are both uniseriate and biseriate. Medullary rays are impregnated with cellular content (starch). Uniseriate medullary rays are having large polygonal cells, which become wider at outer and narrower towards the center. Biseriate medullary rays are elongated cells, wider at the periphery and narrower at the center. Pith is absent in root.

Powder characteristics of leaf

The powder characteristics of leaf are depicted in Fig.14 (a-i). Phloem fibres are lignified with thick wall and narrow lumen. Scleriform type of xylem vessels is found. The leaf powder contains unicellular covering trichomes. Fragments of paracytic stomata with epidermal cells are found. Starch granules are rounded or oval shaped, occurs in single and groups.

Powder characteristic of stem

The following powder characteristics of the stem are shown in Fig.15 (a-i). Starch granules are abundant, both simple and compound (3-6), round and oval in shape. Fragment of pitted, spiral and annular xylem vessels are found. Cortex consists of polygonal parenchymatous cells, more or less isodiametric in surface view.

Powder characteristic of root

In fig. 16 (a-q), fragment of cortex consisting of polygonal and rectangular cells with brownish content were observed. Both lignified and nonlignified lengthy fibres with tapering ends were observed. Non lignified fibres are colourless, thick walled and possess wider lumen than the lignified fibres. The xylem vessels are spiral, annular and pitted thicken. Squarish type of crystals was found. Both simple and compound starch granules were found. Starch granules are oval and round in shape.

Physico-chemical parameters (table **2)**

Ash value

Ash value is a measure of the quality and purity of the drug. The total ash, water soluble ash, acid insoluble ash and sulphated ash of *S. rhombifolia* leaf were determined following standard procedures and were found to be 16 % w/w, 1.2 % w/w, 8 % w/w and 22.5 % w/w respectively.

Total ash of *S. rhombifolia* leaf was found to be more than water soluble ash and acid insoluble ash. Acid insoluble ash was found to be very less as compared to total ash and water soluble ash. Sulphated ash was found to be more than total ash, water soluble ash and acid insoluble ash. The total ash, water soluble ash, acid insoluble ash and sulphated ash of *S. rhombifolia* stem were found to be 6 %w/w, 4 %w/w, 1 %w/w and 8 %w/w respectively. The total ash and water soluble ash of stem powder were found to be more in the drug.

Acid insoluble ash was found to be very less than total ash, water soluble ash and sulphated ash. Sulphated ash was found to be more than total ash, water soluble ash and acid insoluble ash. The total ash, water soluble ash, acid insoluble ash and sulphated ash of *S. rhombifolia* root were found to be 6 % w/w, 3 % w/w, 1 % w/w and 7.5 % w/w respectively. The total ash of the root was found to be more than water soluble ash and acid insoluble ash. Sulphated ash was found to be more than total ash and water soluble ash. Acid insoluble ash was found to be less as compared to other ash values.

Total extractive values

The extractive values are determined to find out the amount of soluble compounds available in a crude drug. Various extractive values of *S. rhombifolia* plant parts were determined using standard procedures. The chloroform, acetone, methanol and water soluble extractive values of leaf of *S. rhombifolia* were found to be 1.2 % w/w, 1.4 % w/w, 3.6 % w/w and 4.8 % w/w respectively. The leaf showed more amount of water soluble component than chloroform, acetone, and methanol soluble components.

The chloroform, acetone, methanol and water soluble extractive values of stem of *S. rhombifolia* were found to be 6 % w/w, 4 % w/w, 8 % w/w and 8.2 % w/w respectively. The stem showed more amount of methanol and water soluble component than chloroform and acetone extract. The chloroform, acetone, methanol and water soluble extractive values of root of *S. rhombifolia* were found to be 1 % w/w, 1.6 % w/w, 1.3 % w/w and 5.66 % w/w respectively. The root showed more amount of water soluble component.

Loss on drying

The moisture content of leaf, stem and root of *S. rhombifolia* were found to be 6.5 %, 9 % and 6 % respectively. The stem of the plant showed more moisture content.

Behavior of powdered materials towards chemical reagent (table 3)

The dried powdered leaf, stem and root of *Sida rhombifolia* were treated with picric acid, concentrated sulphuric acid, hydrochloric acid, nitric acid, glacial acetic acid, 5 % ferric chloride, sodium hydroxide (5 N), potassium hydroxide (5 N), iodine/20 solution and their behaviors against these reagents were observed.

Table 1: Dimension of various leaf constants of *S. rhombifolia*

Parameter	Maximum	Minimum	Average
Stomatal index	33.33	22.22	29.57
Length of stomata	135 µm	75 µm	110.25 µm
Width of stomata	105 µm	45 µm	68.4 µm
Length of stomata pore	120 µm	49.5 µm	71.4 µm
Width of stomata pore	19.5 µm	15 µm	16.16 µm
Palisade cell	11.5	8.25	9.58
Vein-islet	110	20	81.66
Vein termination	60	30	26.66
Length of trichome	930 µm	240 µm	606 µm
Diameter of starch grain in leaf	45 µm	15 µm	30.75 µm
Diameter of starch grain of in stem	37.5 µm	12.5 µm	25 µm
Diameter of starch grain of root	84 µm	12 µm	38.4 µm
Length of phloem fibre in stem	180 µm	72 µm	115.2 µm
Length of phloem fibre in root	192 µm	60 µm	106.8 µm

Table 2: Determination of physico-chemical parameter

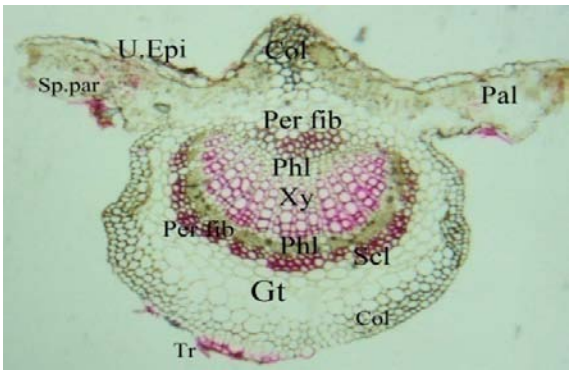
Parts used	Parameter	% w/w
Ash value		
	Total ash	16
Leaf	Water soluble ash	1.2
	Acid in soluble ash	8
	Sulphated ash	22.5
	Total ash	6
Stem	Water soluble ash	4
	Acid in soluble ash	1
	Sulphated ash	8
	Total ash	6
Root	Water soluble ash	3
	Acid in soluble ash	1
	Sulphated ash	7.5
Extractive value		
	Chloroform	1.2
Leaf	Acetone	1.4
	Methanol	3.6
	Water	4.8
	Chloroform	6
Stem	Acetone	4
	Methanol	8
	Water	8.2
	Chloroform	1
Root	Acetone	1.6
	Methanol	1.3
	Water	5.66
Loss on drying		
Leaf	6.5	
Stem	9	
Root	6	

Table 3: Behaviour of powdered leaf, stem and root of *Sida rhombifolia* with chemical reagent

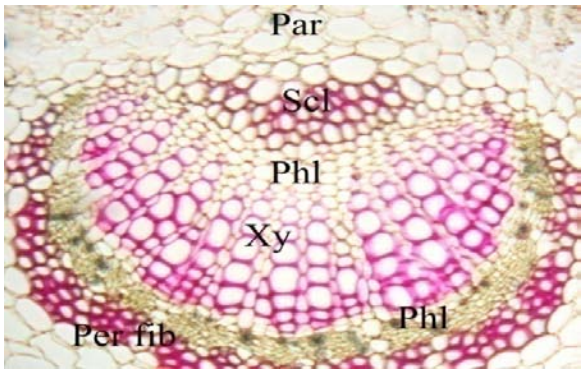
Acid/Reagent	Observation		
	Leaf	**Stem**	**Root**
Powder as such	Light green	Light brown	Light yellow
Powder+Picric acid	Yellow	Yellow	Yellow
Powder+Con. Nitric acid	Light orange	Yellowish orange	Yellowish orange
Powder+Con. HCL	Green	Green	Yellowish green
Powder+Con. H_2SO_4	Deep black	Deep black	Deep black
Powder+Glacial acetic acid	Yellowish green	Light green	Yellowish green
Powder+5 % $FeCl_3$	Light green	Light green	Light green
Powder+NaOH (5 N)	Light green	Yellowish green	Yellowish green
Powder+KOH (5 %)	Green	Yellowish green	Yellowish green
Powder+Iodine/20	Reddish brown	Reddish brown	Reddish brown

Table 4: Fluorescence analysis of powder of *Sida rhombifolia*

Reagent	Leaf		Stem		Root	
	Day light	**Short wave**	**Day light**	**Short wave**	**Day light**	**Short wave**
Powder as such	Light green	Green	Light brown	Brown	Light yellow	Green
Powder+1N NaOH in methanol	Yellowish green	Dark green	Yellowish green	Dark green	Yellowish green	Light green
Powder+1N NaOH	Yellowish green	Light green	Light green	Green	Light green	Green
Powder+Ethanol	Light green	Light green	Yellowish brown	Light green	Yellowish green	Dark green
Powder+HNO_3+NH_3 solution	Green	Light green	Yellowish green	Dark green	Yellow	Green
Powder+50 % HNO_3	Yellowish brown	Light green	Yellowish brown	Dark green	Yellowish brown	Dark green
Powder+1N HCL	Green	Light green	Light yellow	Light green	Yellow	Green
Powder+HCL	Yellowish green	Light green	Watery green	Light green	Yellowish grey	Dark green
Powder+H_2SO_4	Dark brown	Deep green	Brown	Black	Deep brown	Black
Powder+50 % H_2SO_4	Light green	Light green	Yellowish brown	Light green	Yellowish green	Dark green
Powder+Glacial acetic acid	Yellowish brown	Green	Light green	Light green	Watery green	Green
Powder+HNO_3	Yellowish brown	Light green	Yellowish brown	Light green	Yellowish brown	Green



1(a)



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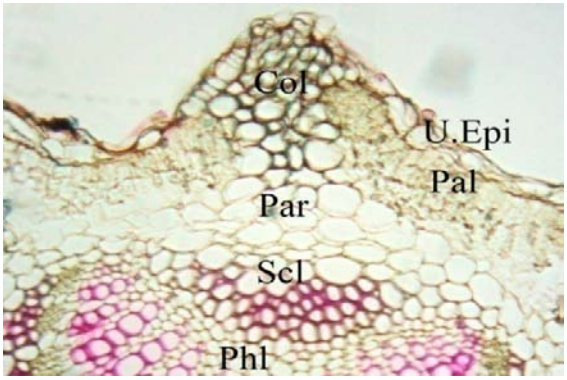
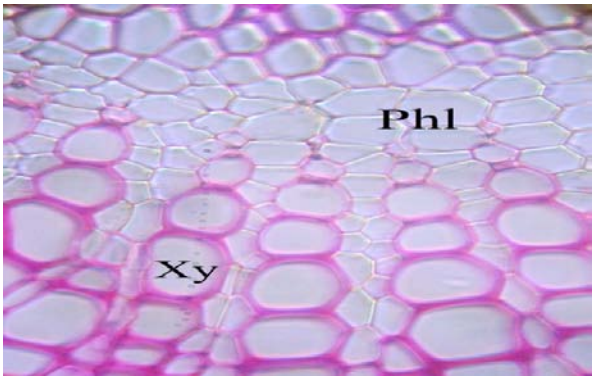
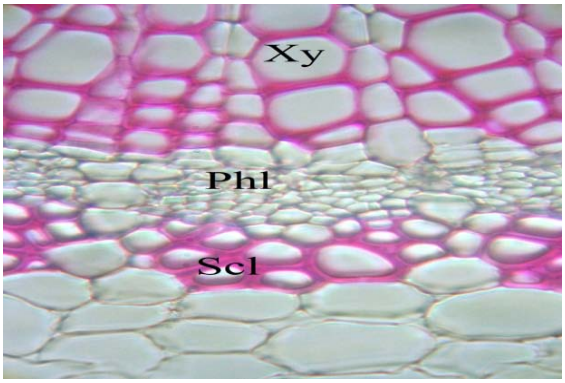


2(b)



3(a)



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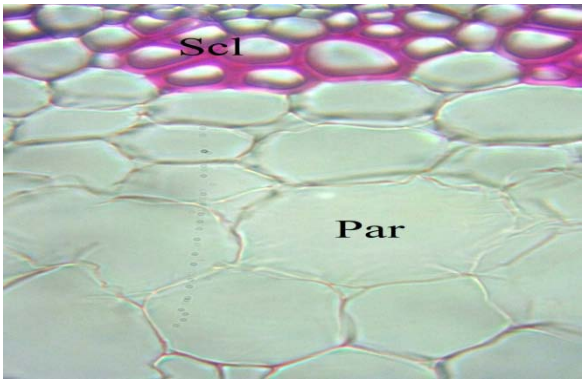
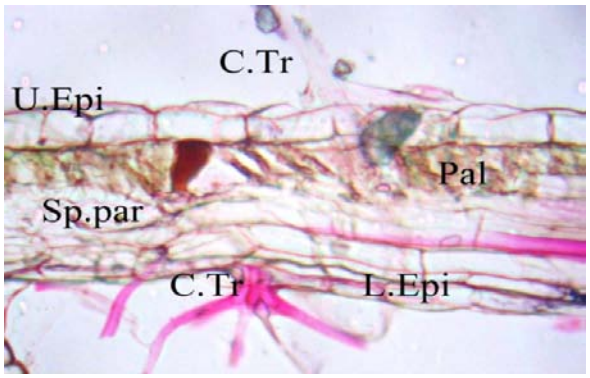


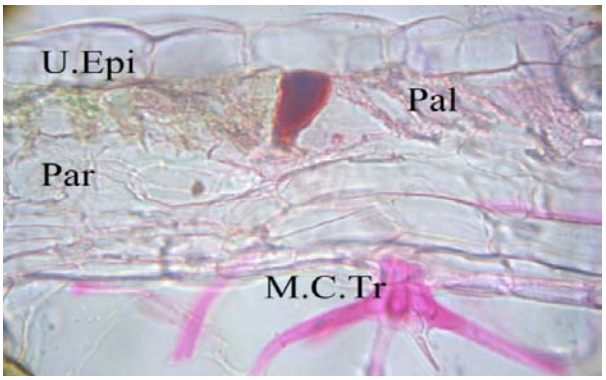
3(c)

Fig. 1(a): Transverse section of leaf at magnification 5x, 5x; fig. 2(a, b): Transverse section of leaf at magnification 5x, 10x; fig. 3(a, b, c): Transverse section of leaf at magnification 5x, 45x

Abbreviations: U. Epi-Upper epidermis, Col-Collenchyma, Par-Parenchyma,Scl-Sclerenchyma, Tr-Trichomes, Gt-Ground tissues, Per fib-Pericyclic fibre, Sp par-Spongy parenchyma, Pal-Palisade, Xy-Xylem, Phl-Phloem.



3(a)



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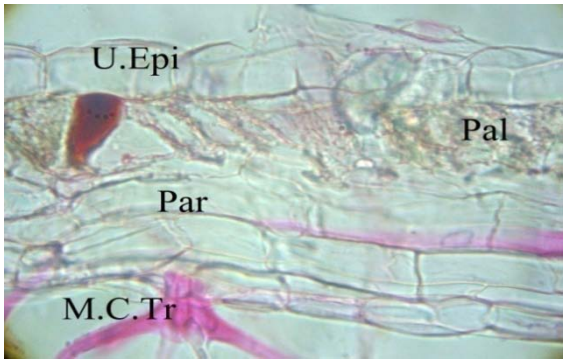

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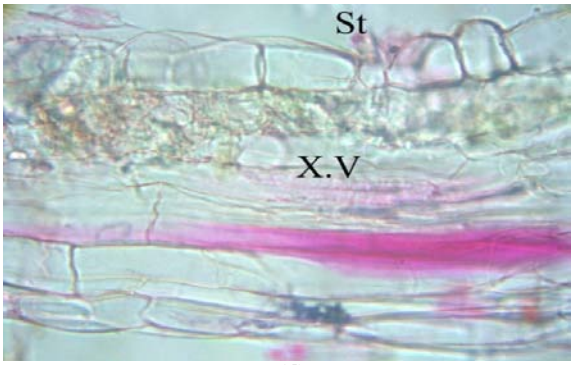
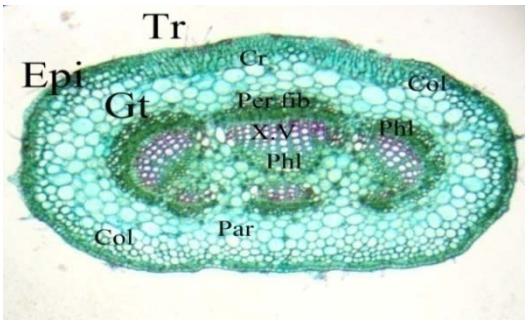


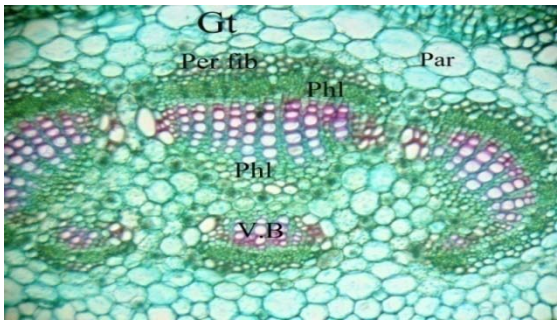
3(d)

Fig. 3(a, b): Transverse section of lamina at magnification 5x, 10x; fig. 3(c, d): Transverse section of lamina at magnification 10x, 45x

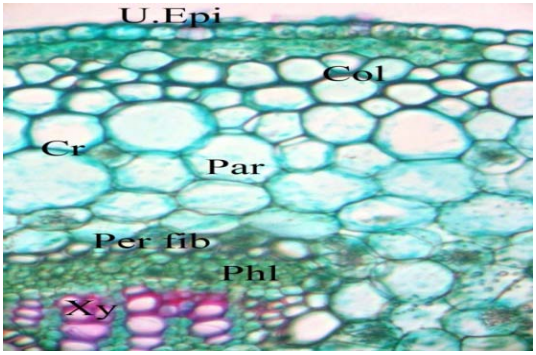
Abbreviations: U. Epi-Upper epidermis, C. Tr-Trichomes, M. C. Tr-Multicelluar covering trichomes, Par-Parenchyma, Pal-Palisade, St-Stomata, X. V-Xylem vessel, L. epi-Lower epidermis.



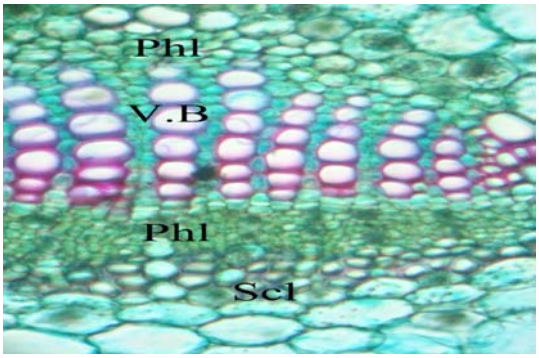
4(a)



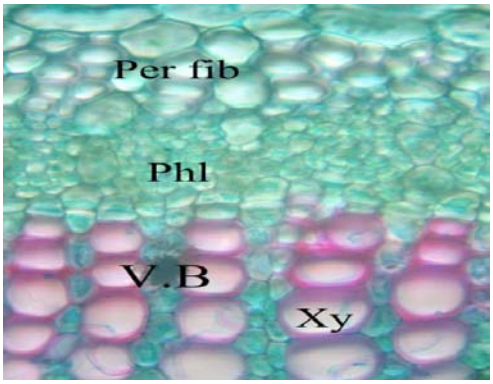
5(a)



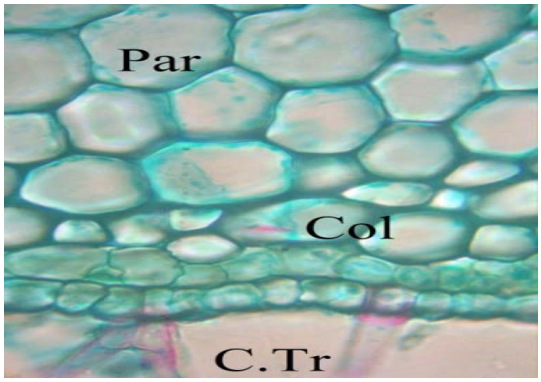
6(a)



6(b)



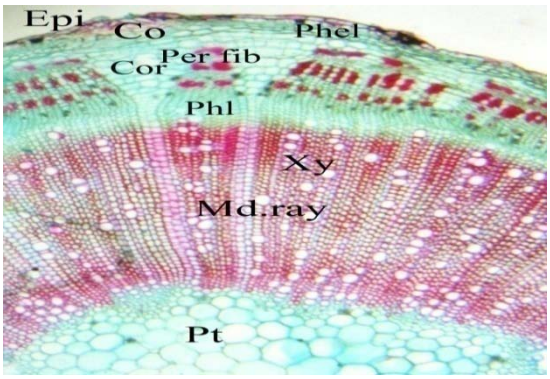
7(a)



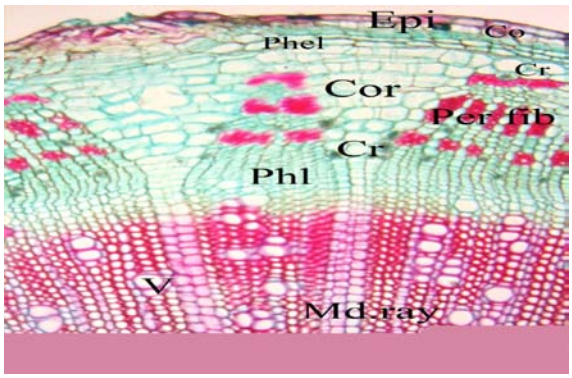
7(b)

Fig. 4(a): Transverse section of petiol at magnification 5x, 5x; fig. 5(a): Transverse section of petiol at magnification 5x, 10x; fig. 6(a, b): Transverse section of petiol at magnification 10x, 10x; fig. 7(a, b): Transverse section of petiol at magnification 5x, 45x

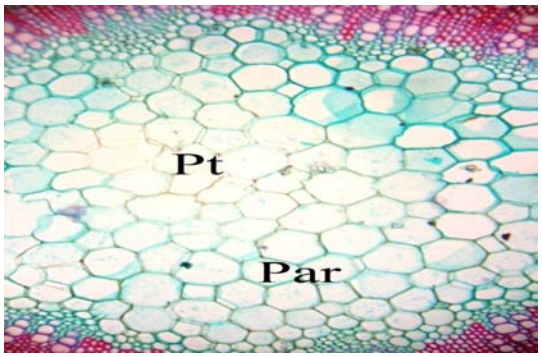
Abbreviations: Epi-Epidermis, Col-Collenchyma, Gt-Ground tissue, Tr-Trichomes, C. Tr-Covering trichomes, Cr-Crystal, Par-Parenchyma, Scl-Sclerenchyma, Phl-Phloem, Xy-Xylem, Par fib-Pericycle fibre, Xy-Xylem, V. B-Vascualr bundle.



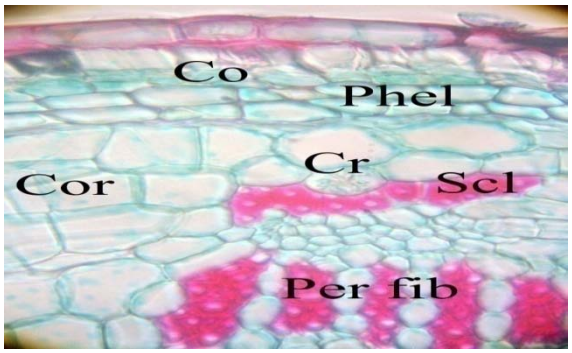
8(a)



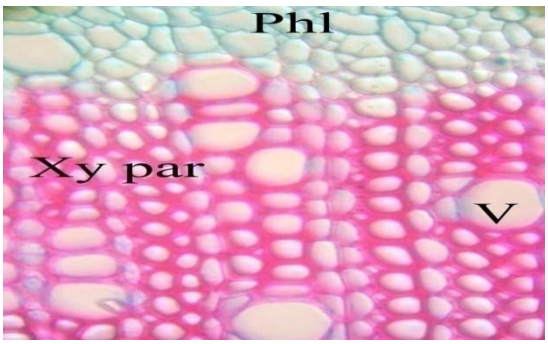
9(a)



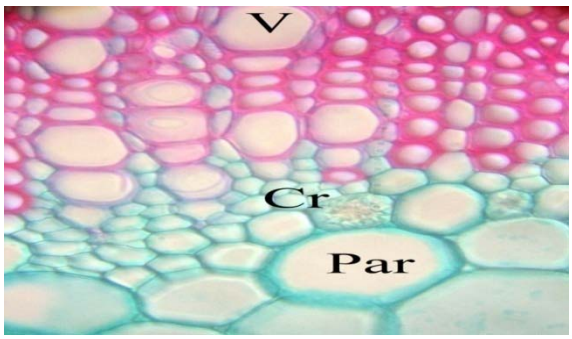
9(b)



10(a)



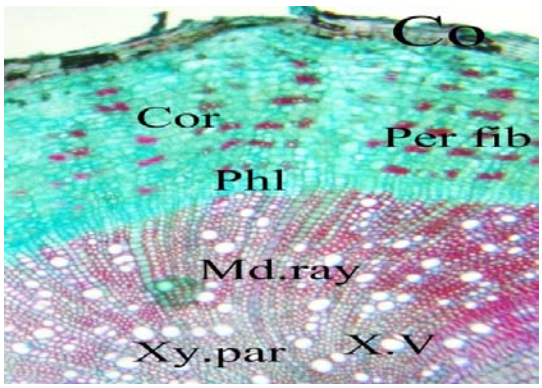
10 (b)



10(c)

Fig. 8(a): Transverse section of stem at magnification 5x, 5x; fig. **9(a, b): Transverse section of stem at magnification 5x, 10x;** fig. **10(a, b, c): Transverse section of stem at magnification 5x, 45x**

Abbreviations: Epi-Epidermis, Co-Cork, Cor-Cortex, Cr-Crystal, Par-Parenchyma, Scl-Sclerenchyma, Phl-Phloem, Xy-Xylem, Per fib-Pericycle fibre, Xy-Xylem, V-Vessel, Md ray-Medullary rays, Pt-Pith



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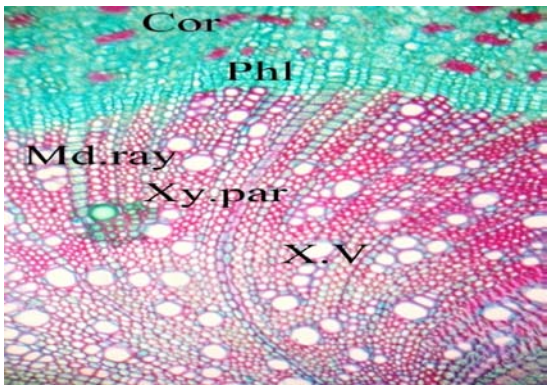
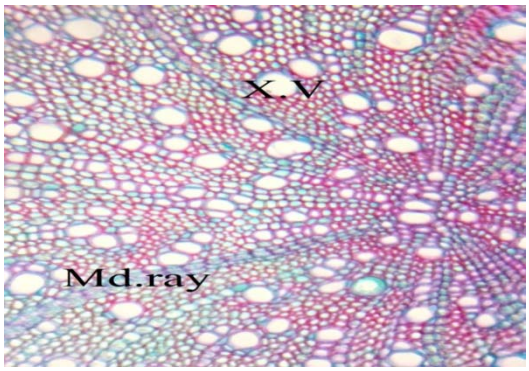
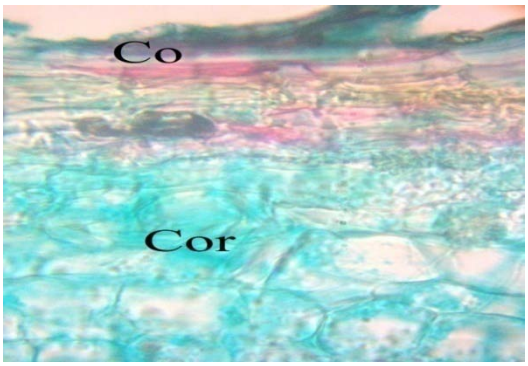


12 (a)



12 (b)



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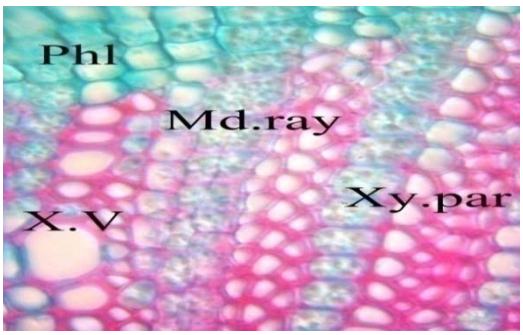
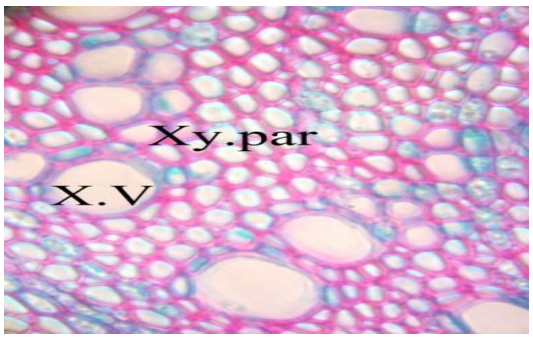


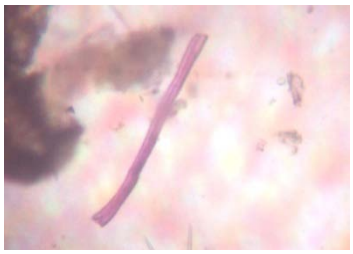
13(b)



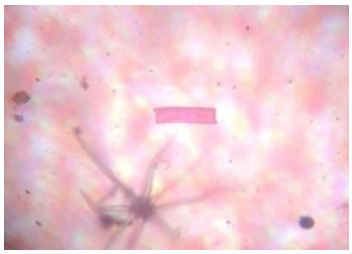
13(c)

Fig. 11(a): Transverse section of root at magnification 5x, 5x; fig. **12(a, b): Transverse section of root at magnification 5x, 10x;** fig. **13(a, b, c): Transverse section of root at magnification 5x, 45x**

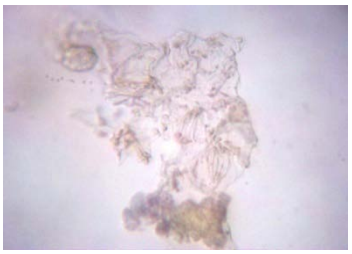
Abbreviations: Co-Cork, Cor-Cortex, Par-Parenchyma, Phl-Phloem, Xy-Xylem, Par fib-Pericycle fibre, Xy-Xylem, X V-Xylem vessel, Md ray-Medullary rays.



14(a)



14(b)



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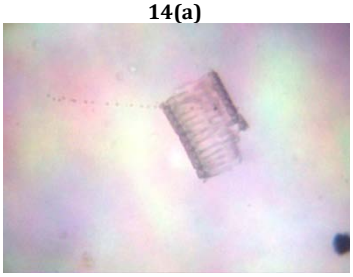
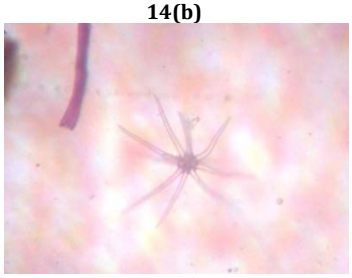
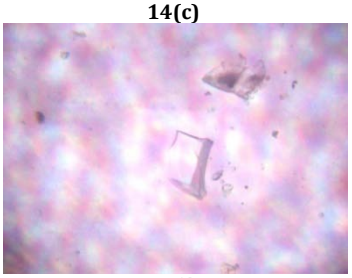


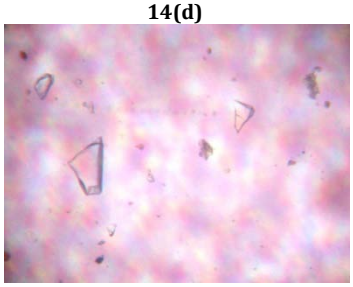
14(d)



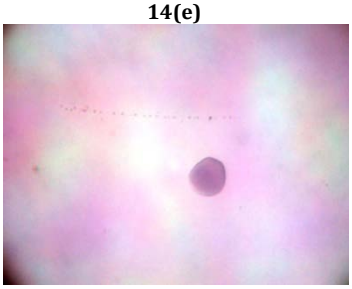
14(e)



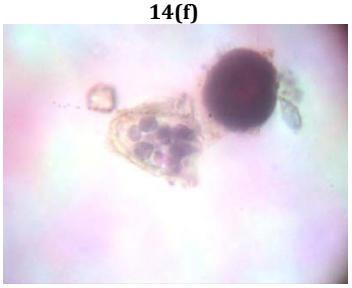
14(f)



14(g)

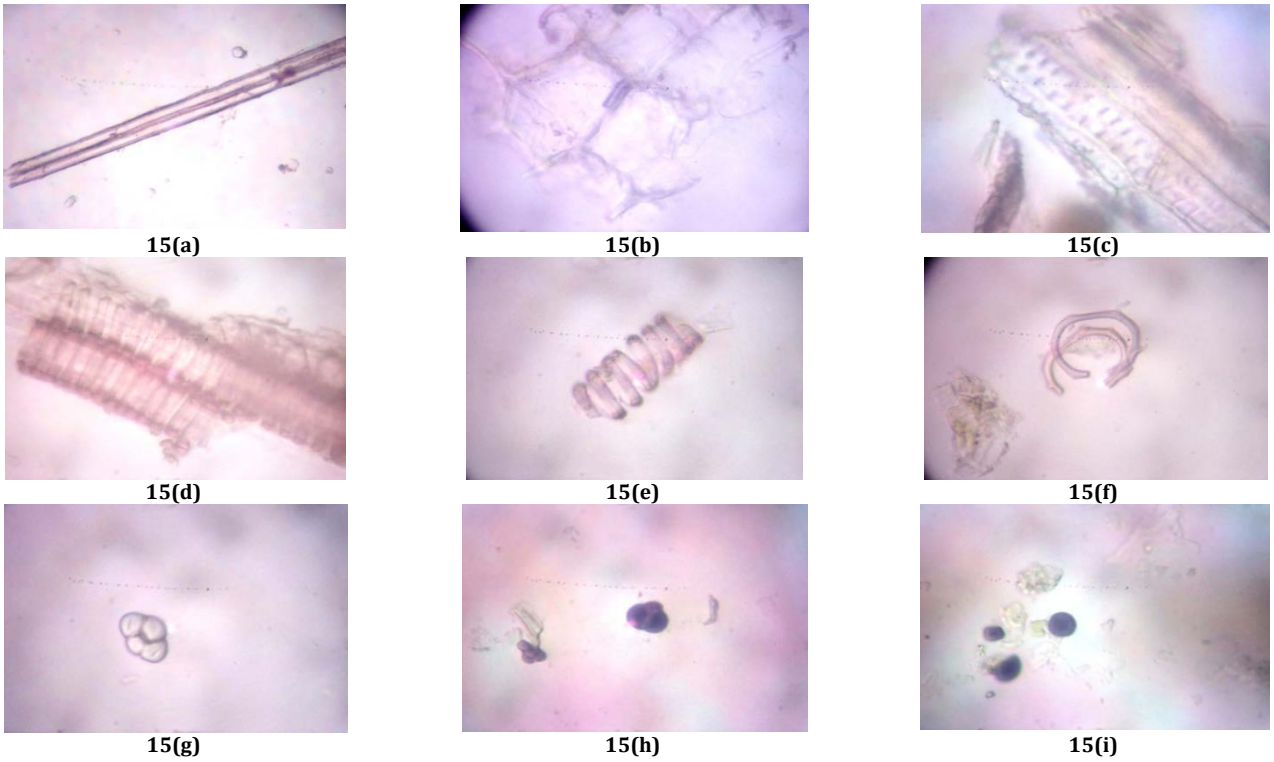


14(h)



14(i)

Fig. 14: Powder Microscopy of Leaf, (a,b):Phloem fibre, (c): Stomata, (d): Xylem vessels, (e): Trichomes, (f, g): Crystal, (h, i): Starch granules at **5x, 45x**



15(a) 15(b) 15(c)

15(d) 15(e) 15(f)

15(g) 15(h) 15(i)

Fig. 15: Powder Microscopy of Stem, (a): Fibre, (b): Cortex, (c, d, e, f): Tracheal element, (g, h, i): Starch granules at 5 x, 45 x

16(a) 16(b) 16(c) 16 (d)

16(e) 16(f) 16(g) 16(h)

16(i) 16(j) 16(k) 16(l)

16(m) 16(n) 16(o) 16(p)

Fig. 16: Powder characteristic of root, (a,b,c): Cork cell, (d,e,f): Cortex, (g,h): Fibre, (I,j,k,l): Xylem vessels, (m,n,o): Crystals, (p,q): Starch granules at 5 x, 45 x

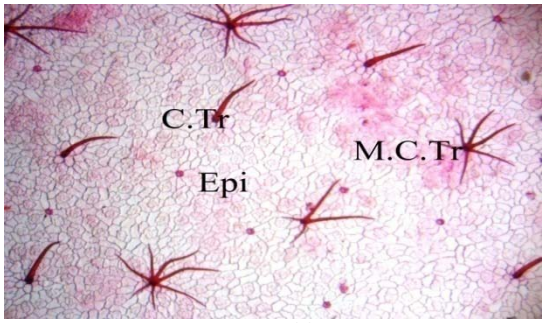


Fig. 17(a)

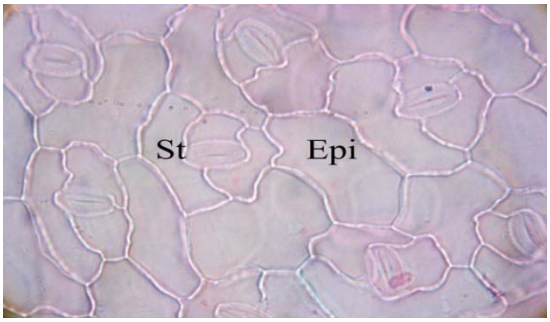


Fig. 17(b)

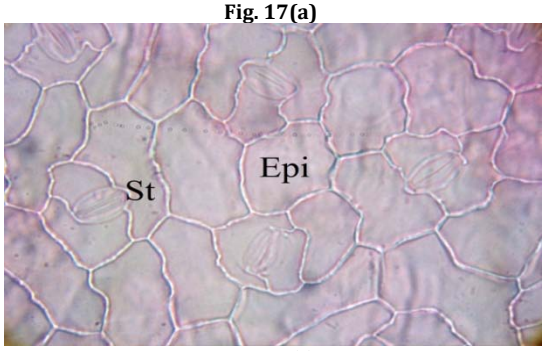


Fig. 17(c)

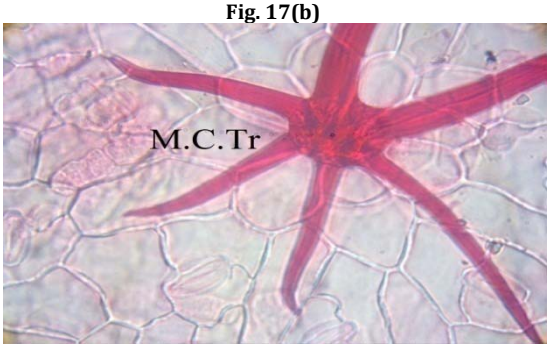


Fig. 17(d)

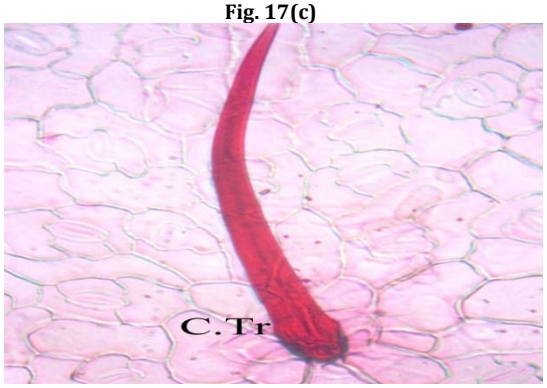


Fig. 17(e)

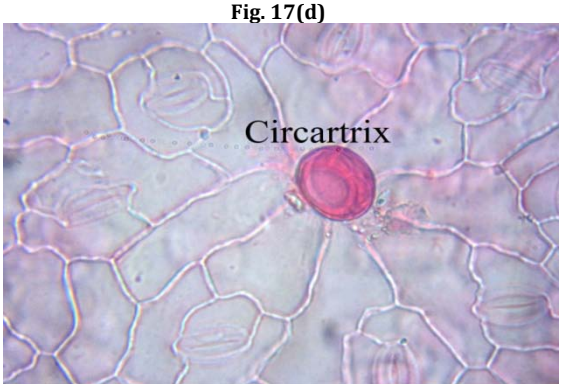


Fig. 17(f)

Fig. 17: Quantitative Microscopy of leaf, (a): Epidermal cells with covering trichomes, (b,c): Epidermal cells with anisocytic/cruciferous stomata, (d): Epidermal cells with multicellular branched covering trichomes(stellate trichomes). (e): Epidermal cells with covering trichomes, (f): Epidermal cells with covering circartrix

Fluorescence analysis of powder of *Sida rhombifolia* (table 4)

Fluorescence analysis of leaf, stem and root powder of *Sida rhombifolia* has been carried out in day light and under UV light (256 nm). The powders were treated with differing organic solvents and reagents and the fluorescence was observed in normal day light and under UV light.

DISCUSSION

The systematic informations on medicinal plant in respect to its botanical identification and physico-chemical characters may be useful for pharmacognostical study and standardization of herbal drugs used for traditional practice. It may be treated as the therapeutic diagnostic tool for the scientists who are sincere in evaluating the herbal medicines of indigenous source.

Adulterated or inferior medicinal plants may cause severe health problems when taken by the consumers and this may lead to legal problems in the pharmaceutical industries. To maintain the quality of raw materials and finished products of crude drugs, the authentification of source, microscopical and physico-chemical studies followed by sophisticated modern instrumental techniques provide more reliability [22].

The anatomical study of medicinal plants is a major aid for the authentification of drugs especially important for identification of powdered drugs because in these cases, most of the morphological diagnostic features are lost [23].

Microscopical evaluation serves as one of the simplest and cheapest methods for the correct identification of medicinal plants. Single layer of straight walled rectangular cells in upper epidermis followed by 6-7 layers of collenchymatous cell and thin layer of parenchymatous cell of about 4-5 layer, single layer of polygonal parenchymatous cell in lower epidermis, collateral arc shaped vascular bundle surrounded by pericycle fibre in mid rib of leaf; presence of unicellular covering trichomes and opening of stomata, stellate (star shaped) multicellular covering trichomes in leaf lamina are the various diagnostic characters of *Sida rhombifolia* leaf. The compound starch granules, pitted, spiral and annular xylem vessels in stem, cortex of polygonal and rectangular cells with brownish content, both lignified and non lignified fibres and xylem vessels of spiral, annular thickening and squarish type of crystals in root are also the important features for identification and authentification of this drug.

The ash value is important to find out the presence or absence of foreign inorganic matter such as metallic salts, earthy matter and other impurities. The extractive values are useful to detect exhausted or adulterated drug. Preliminary phytochemical screening will reveal the information of nature of chemical constituents present in the drug. Preliminary phytochemical

screening revealed the presence of alkaloids, glycosides, steroids, flavonoids, terpenoids, saponins, protein and aminoacids in *Sida rhombifolia* plant. The ultra violet light produces fluorescence in many natural products which is not visible in daylight. The powder crude drug does not produce fluorescence on its own, but it may be converted into fluorescent derivatives in presence of different solvents and reagents. Hence, this fluorescence study could be one of the parameter for qualitative evaluation of crude drugs [24].

CONCLUSION

In this study, the microscopical as well as physico-chemical characteristics of various parts of the plant *Sida rhombifolia* were investigated. The resulted data could be useful to differentiate closely related plant species having similar phytoconstituents and pharmacological activities. Further, these data could be helpful in proper identification of the subjected plant from its allied species and particularly if its parts are available in powder form.

CONFLICT OF INTERESTS

Declared None

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