Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 934-942

ISSN: 0975-4873

Research Article

Development of Quality Control Parameters for Standardization of *Triumfetta rhomboidea*

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Available Online:3rd September, 2015

ABSTRACT
Triumfetta rhomboidea is a herbaceous perennial plant found abundantly throughout India. The plant is used in the treatment of dysentery, diarrhoe, ulcer, leprosy and gonorrhoea. The present study comprises taxonomic details, macro and microscopical characters of parts used, and physico-chemical details. This study will be helpful in setting some pharmacopoeial standards and preparation of monograph of this plant.

Key words: *Triumfetta rhomboidea*, Microscopical character, Powder microscopy, Physico-chemical study.

INTRODUCTION

The herbal drugs can be used safely if their standard parameters such as authentification, safety, efficacy and quality are maintained as per specifications. In view of the growing demand and use of herbal drugs, it is crucial to ascertain standard samples of herbal drugs for future reference[1]. In recent years, the rapid development of herbal drugs provides a systematic approach to evaluate these drugs in modern pharmacognosy by qualitative and quantitative means[2].

Nowadays, analytical instruments are playing an important role in the production and evaluation of new herbal for the consumers and the environment. The use of sophisticated instruments is a fascinating measure of chemical analysis. Though, several modern instrumental techniques are required to solve analytical problems still the value of classical methods can't be over looked. Therefore, it is necessary to perform some physical and chemical operations on the drug sample prior to the actual analysis.

Triumfetta rhomboidea (family-Tiliaceae) is a herbaceous perennial plant. It is distributed throughout tropical and subtropical India[3]. It is 0.6 to 1.5m in height; branches are slender, more or less pubescent with simple hairs. Leaves are variable, stipulate, 3-lobed, irregularly serrate, clothed with simple and stellate hairs on both surfaces. Flower is yellow in colour, dense terminal and leaf opposed cymes. Buds are oblong apiculate, peduncles and pedicels are very short; bracts are subulate. Sepals are oblong, hooded and apiculate at the apex. Petals are shorter than the sepals, obovate-oblong and ciliate at the base. Fruits are 4mm in diameter, pubescent and spines are glabrous[4].

The leaf, bark, root, flower and fruit of the plant are traditionally used for treatment of various diseases. In Ayurveda the root is bitter and acrid; aphrodisiac, tonic, cooling; useful in dysentery. The leaves and stem are used as a poultice on tumor[5]. Powdered leaf infusions of *Triumfetta rhomboidea* are drunk represents for the treatment of anemia in different regions of East Africa[6]. Triumfetta(Tiliaceae) species are used in the folk medicine forthe treatment of various diseases, such as diabetes, leprosy, diarrohea, demulcent, etc. *Triumfetta rhomboidea* is locally used as antidiabetic, aphrodisiac, tonic, galactogenic, roots asdiuretic, barks in diarrheoa, leaves and flowers as astringent[7]. Pounded roots are given in the treatment of intestinal ulcer. Leaves, flowers and fruit are mucilaginous demulcent, astringent, and also used in gonorrhoea and against leprosy[8].

MATERIALS AND METHODS

Collection and preparation of specimen

The plant specimens for the proposed study were collected from Bargarh, Odisha. The required sample of different organs were cut and remove from the plant and fixed on FAA (Formalin-5ml + Acetic acid-5ml +70% Ethyl alcohol-90 ml) after 24 hrs of fixing, the specimen were dehydrated with graded series of tertiary-butyl alcohol as per the scheduled given by Sass,1940. Infiltration of the specimen was carried by gradual addition of paraffin wax (melting pointing 58^0 to 60^0C) until solution attained super saturation. Then the specimen was cast into paraffin wax blocks.

Sectioning and staining

The paraffin embedded specimen was sectioned with help of Rotary Micrometer. The thickness of the specimen was 10-12µm[9]. The section was stained with safranin ana fast green. The dye render deep red color to lignin and bright green color to cellulose[10]. For studying the stomatal

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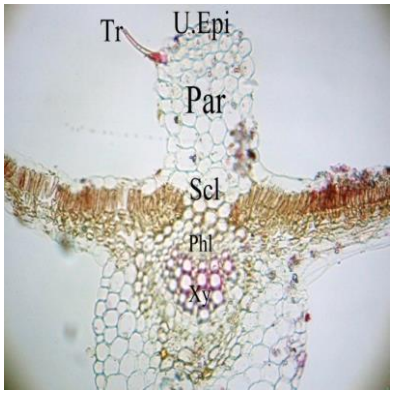
Table 1: Dimention of various leaf constants of *Triumfetta rhomboidea*

Parameter	Maximum	Minimum	Average
Stomatal index	50	30	38.28
Length of stomata	120 µm	97.5 µm	109.5 µm
Width of stomata	75 µm	52.5 µm	68.25 µm
Length of stomata pore	75 µm	52.5 µm	63 µm
Width of stomata pore	22.5 µm	15 µm	15.75µm
Palisade cell	11.5	8.25	9.58
Vein-islet	40	30	35
Vein termination	110	70	95
Length of trichome	900 µm	225 µm	555.75 µm
Diameter of starch grain of stem	45 µm	22.5 µm	29.62 µm
Diameter of starch grain of root	75 µm	22.5 µm	38.25 µm
Length of phloem fibre in stem	525 µm	105 µm	310.8 µm
Length of phloem fibre in root	525 µm	120 µm	327 µm



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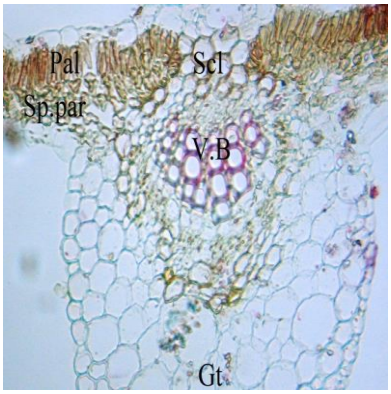

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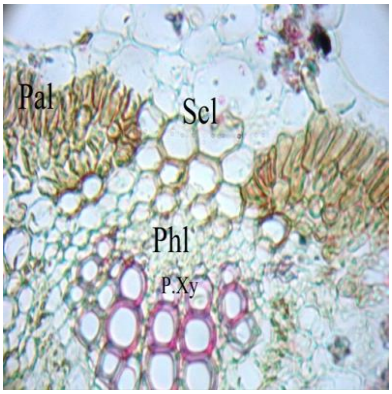

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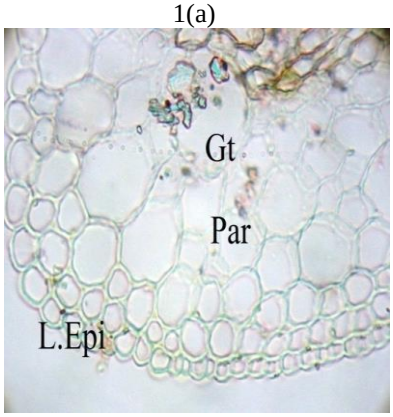

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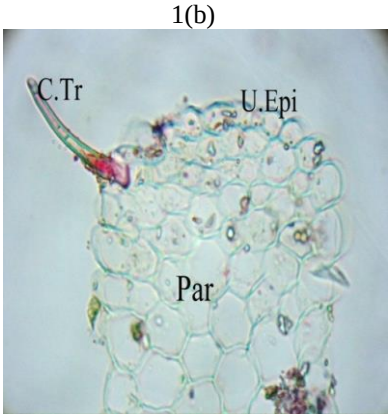

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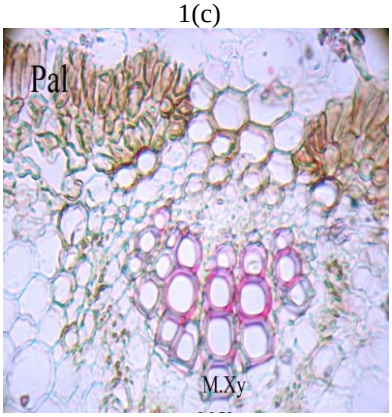


1(f)

Figure 1(a): Transverse section of leaf at magnification 5x, 10x; Figure 1(b): Transverse section of leaf at magnification 10x, 10x., Figure (c, d, e &f): Transverse section of leaf at magnification 5x, 45x.
Abbreviations: U.Epi-Upper epidermis, Par-Parenchyma, Phl-Phloem Scl-Sclerenchyma, C.Tr-Covering trichome, Gt-Ground tissues, Pal- Palisade cells, Sp. par- Spongy parenchyma, P.xy-Proto xylem, M.xylem-Meta xylem

morphology, venation pattern and trichome distribution, paradermal section as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by maceration Jeffrey's maceration fluid were prepared[11].

Photomicrographs

Photographs of different magnifications were taken with Nikon Labphoto 2 microscopic unit. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structure have birefringent property under polarized light. They appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books[12].

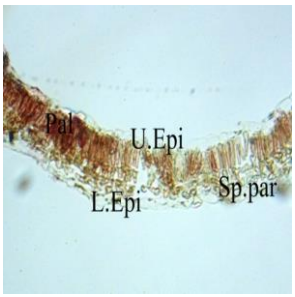
Quantitative determination was carried out for measurement of diameter of starch grain and length of phloem fibre[13], physico-chemical studies[12], behavior of powder drug towards different chemical reagent[14], fluorescence analysis[15].

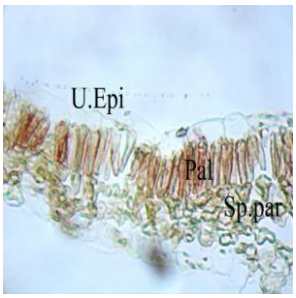
Preliminary phytochemical screening

Preliminary phytochemical screening was carried out using the standard methods[16].

RESULTS

Quantitative microscopy





2(a) 2(b)

Figure 2(a): Transeverse section of lamina at magnification 5x,10x

Figure 2(b): Transeverse section of lamina at magnification 5x,45x

Abbreviation: U.Epi-Upper epidermis, L.Epi-Lower epidermis. Sp par-Spongy parenchyma. Pal-Palisade cell.

Epidermis is wavy walled and also straight walled polygonal. It shows the presence of anomocytic and paracytic stomata. Epidermal surface shows presence of both glandular and covering trichonmes. Covering trichomes are uniseriate, unicellular, thick wall with acute apex. It shows the presence of stellate trichomes. Vein-islet and vein termination are prominent. Veins are lignified.

Macroscopical characters

Leaves are **g**reen in colour with characteristic taste and slight odour. The colour of stem is brown and taste is bitter with slight odour.The roots are found light brown in colour with characteristic taste.

Microscopical study of leaf

Upper epidermis is single layered, polygonal parenchymatous cells. Covering trichomes are present above the epidermal cell. Epidermal layer is followed by 9-10 layers of parenchymatous cell. Vascular bundle is arc shaped and is more towards dorsal side. It is bicollateral (xylem is surrounded by phloem). Xylem is lignified and phloem is non-lignified. Vascular bundle is surrounded by pericycle fibre. Pericycle consists of sclerenchymatous cells which are highly lignified. Vascular bundle is surrounded by ground tissue. It consists of thin walled parenchymatous cell (round or polygonal). Some are small in size and some are bigger in size. Ground tissue covers the dorsal and ventral side.

Transeverse section of lamina

Lamina is dorsiventral in nature. Upper epidermis is single layer and consists of polygonal cell. Covering trichomes are present on the epidermis. Mesophyll region contain palisade cells and spongy parenchymatous cells. Palisade cells are single layered, compactly arranged, radially elongated cells containing brownish matter. Palisade cells are followed by spongy parenchyma. It consists of 3-4 layer of loosely arranged parenchymatous cells. Lower epidermis is similar to upper epidermis.

Transeverse section of petiol

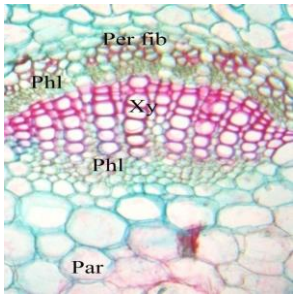
Epidermis is singly layered, tangentially elongated, not covered by cuticle. These are polygonal cells contain covering trichomes. Epidermal layer is followed by cortex zone. Below the epidermis about two layer of thick walled collenchymatous cells are present. Collenchymatous cell is followed by parenchymatous cell. Smaller and bigger cells are present towards inner side. Few cells contain clusture of crystals. Five number of vascular bundle are present, out of which one vascular bundle is bigger than others. Three vascular bundle are fully developed and two are less developed. Xylem is lignified and phloem is non lignified. Xylem is endarch (protoxylem lies towards centre and meta xylem lies towards periphery). Growth of xylem is centrifugal. Arrangement of vascular bundle is bicollateral. Pericycle fibre consists of sclerenchymatous cell, which are lignified and are arranged as crown above phloem region of vascular bundle. At the centre pith is present. It consists of large thin walled parenchymatous cells.

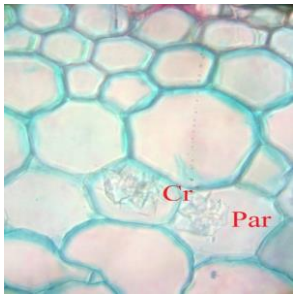
Transeverse section of bark

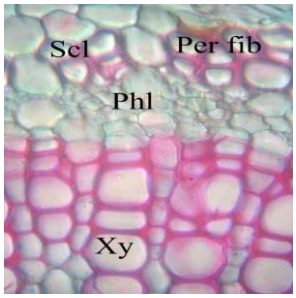
Cork cell is about 2-3 layers, consists of radish brown matter. The cells are oval to rectangular shapes. It is followed by stratified cortex. These are thin walled parenchymatous cells of rectangular shapes. It consists of many layers of parenchymatous cell , extend to the next region. It contains squarish / prismatic calcium oxalate crystals. Group of pericycle fibres are appeared in the cortex region. Each group contains approximately 9-16 of sclerenchymatous cells. These are highly lignified when treated phloroglucinol and concentrated hydrochloric acid. Group of pericycle fibres become more diverge towards the centre which gives the appearance of divergent rays.

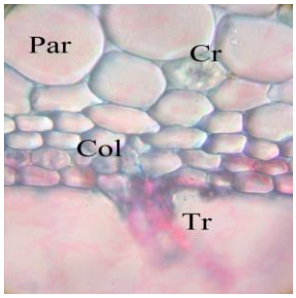
Transeverse section of stem

Cork consists of few layer of irregular shape and contain reddish brown matter. Below the cork region stratified





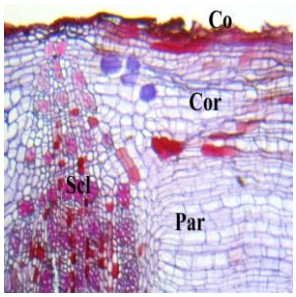


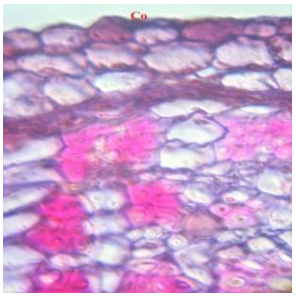


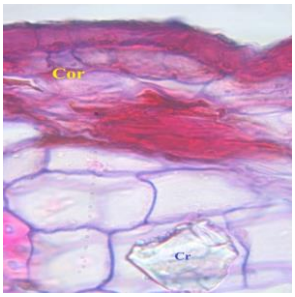
3(a) 3(b) 3(c) 3(d)

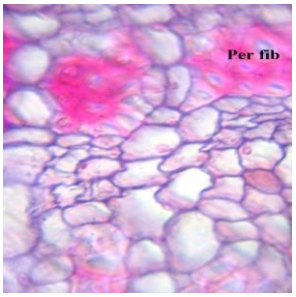
Figure 3(a): Transverse section of petiol at magnification 10x,10x; Figure 3(b, c & d): Transverse section of petiol at magnification 5x, 45x.

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





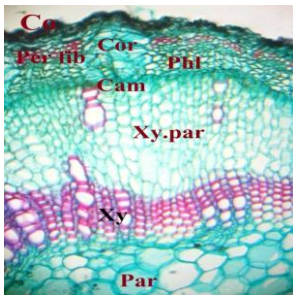


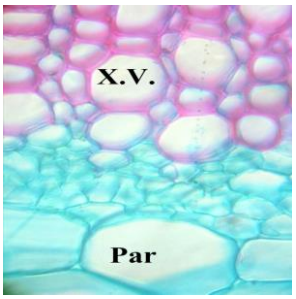


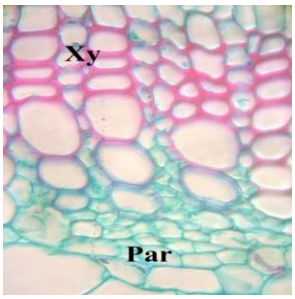
4(a) 4(b) 4(c) 4(d)

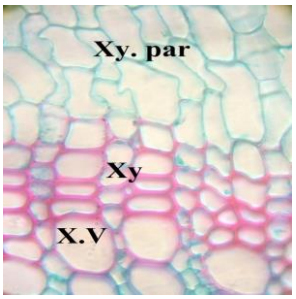
Figure 4(a): Transverse section of stem at magnification 5x, 10x; Figure 4(b, c & d): Transverse section of stem at magnification 5x, 45x.

Abbreviations: Epi-Epidermis, Co-Cork, Cor-Cortex, Cr-Crystal, Par-Parenchyma, Scl-Sclerenchyma, Per fib-Pericycle fibre.





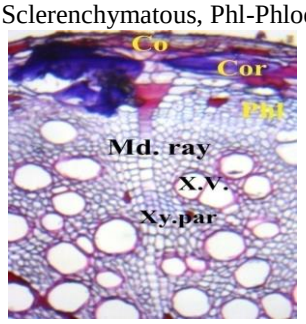


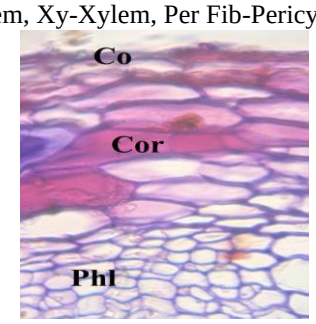


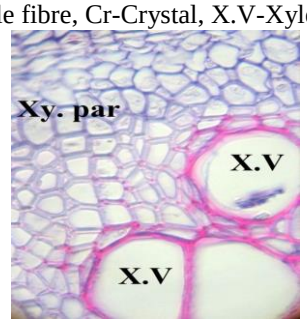
5(a) 5(b) 5(c) 5(d)

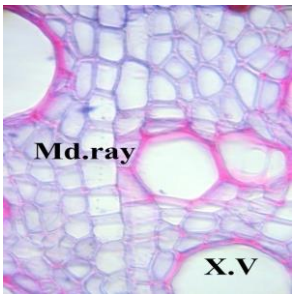
Figure 5(a): Transverse section of stem at magnification 5x, 10x; Figure 5(b,c & d): Transverse section of stem at magnification 5x,450x.

Abbreviation: Co-Cork, Cor-Cortex,, Cam-Cambium, Xy par-Xylem parenchyma, , Par-Parenchyma, Scl-Sclerenchymatous, Phl-Phloem, Xy-Xylem, Per Fib-Pericycle fibre, Cr-Crystal, X.V-Xylem vessel.





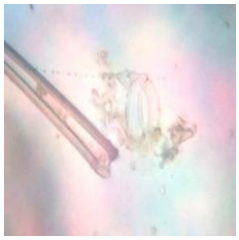


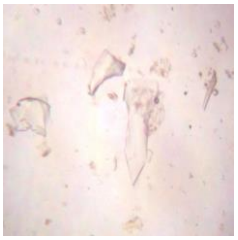


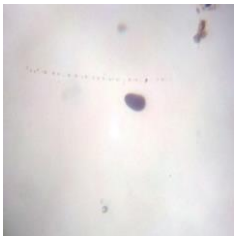
6(a) 6(b) 6(c) 6(d)

Figure 6(a): Transverse section of root at magnification 5x, 10x; Figure 6(b): Transverse section of root at magnification 10x, 10x; Fig. 6(c& d): Transverse section of root at magnification 5x, 45x

Abbreviation: Co-Cork, Cor-Cortex, Phl-Phloem, Md.ray-Medullary rays, X.V-Xylem vessel, Xy.par-Xylem parenchyma.



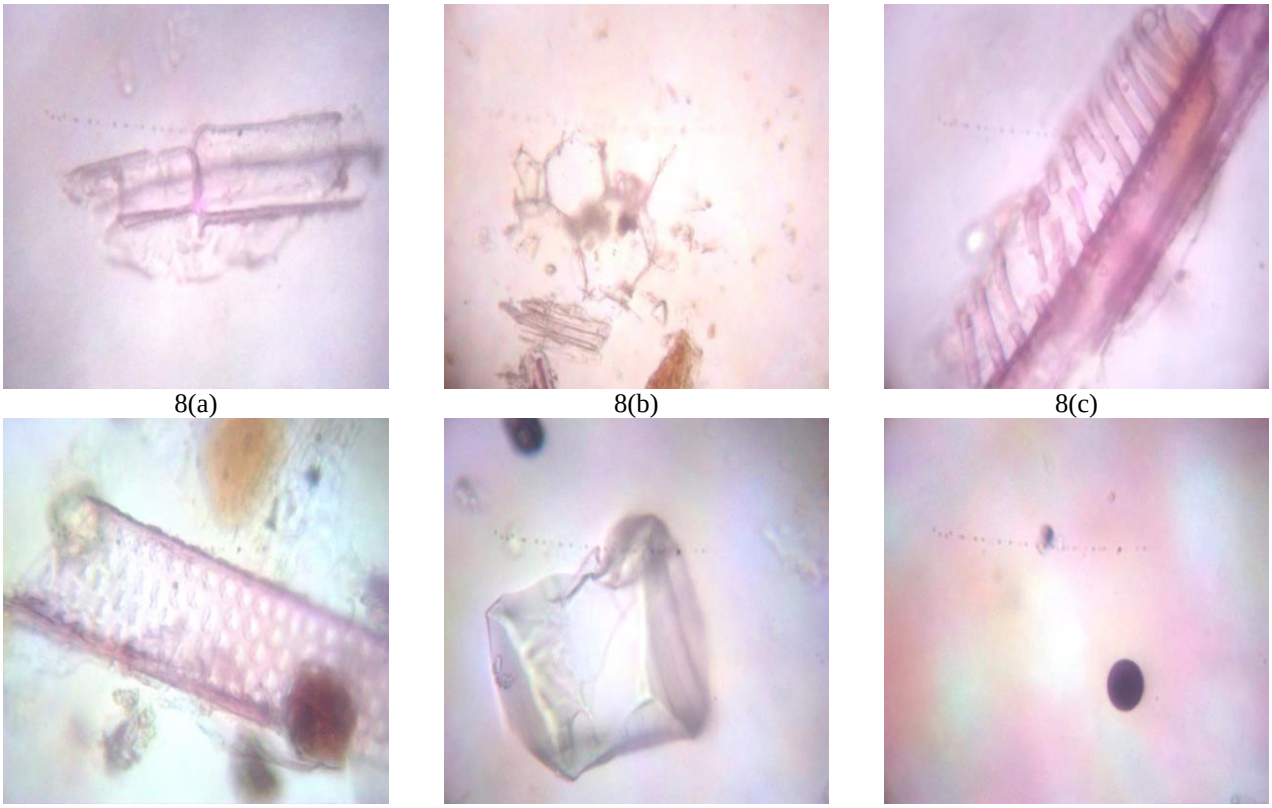




7(a) 7(b) 7(c) 7(d) 7(e)

Figure 7: Powder Microscopy of Leaf, Figure 7(a &b): Trichomes
Figure 7(c): Stomata; Figure 7(d): Crystal, Figure 7(e): Starch granule

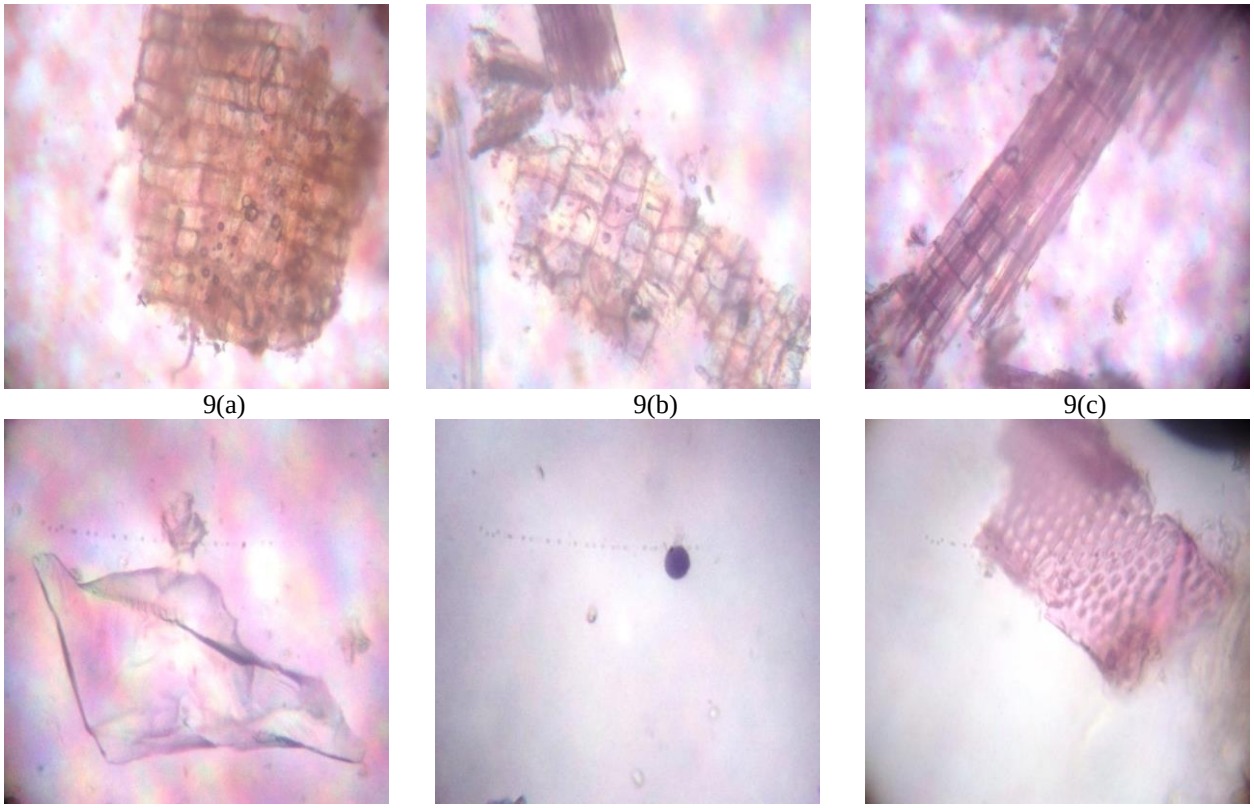
cortex is present. These are large thin walled parenchymatous cells. The cells contains clusture of calcium oxalate crystals. Cambium is present in between cortex and vascular bundle. It is about 2-3 layers and consists of rectangular cells. Vascular bundle consists of xylem and phloem. It open, due to presence of cambium.



8(a) 8(b) 8(c)

8(d) 8(e) 8(f)

Figure 8: Powder Microscopy of Stem, (a): Fibre, (b): Cortex, (c,d,e,f): Tracheal element, (g,h,i): Starch granules.



9(a) 9(b) 9(c)

9(d) 9(e) 9(f)

Figure 9: 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

Table 2: Determination of physico-chemical parameters

Parts used	Parameter	%w/w
Ash value		
	Total ash	10.33
Leaf	Water soluble ash	4
	Acid in soluble ash	2.2
	Sulphated ash	12.2
	Total ash	7
Stem	Water soluble ash	3
	Acid in soluble ash	1.5
	Sulphated ash	11.5
	Total ash	7
Root	Water soluble ash	4.5
	Acid in soluble ash	4
	Sulphated ash	11
Extractive value		
	Chloroform	3.2
Leaf	Acetone	5.6
	Methanol	19.2
	Water	32
	Chloroform	3.2
Stem	Acetone	0.4
	Methanol	1.4
	Water	5
	Chloroform	1
Root	Acetone	0.66
	Methanol	2.6
	Water	6
Loss on drying		
Leaf	9.5	
Stem	10	
Root	7.5	

Arrangement of vascular bundle if collateral. Xylem is lignified. Xylem vessels are large in size. Xylem is surrounded by thin walled non lignified xylem parenchyma. Phloem is present as group above the xylem parenchyma. Phloem is nonlignified. Patches of pericycle fibre are present above the vascular bundle. Each group contains about 9-16 no of sclerenchymatous cell. Medullary rays is absent. Pith occupies large portion at the centre. It consists of thin walled polygonal parenchymatous cells. Cells are closely arranged without having intercellular spaces.

Transeverse section of root

Cork consists of 2-3 layers of tabular cells, irregular shape. Cork region followed by cortex. It consists of 3-4 layers of slightly flattened parenchymatous cells. Cells contain reddish brown matter. Vascular bundle is present below cortex. Arrangement of vascular bundle is collateral. Xylem is lignified and phloem is non lignified. Xylem vessels may be spiral, annular, scleriform, reticulat or pitted. Most of the vessels are bigger in size. Most of the xylem vessels are singly and large while few xylem vessels are compound. Vessels are surrounded by xylem parenchyma. Phloem is present above the xylem and is 7-8 layer closely arranged. It consists of thin walled parenchymatous cells of irregular shape. Medullary rays are multiserriate. Medullary rays narrows towards centre and becomes diverge towards periphery. It consists of squarish and rectangular shape of parenchymatous cell. Pith is absent.

Powder characteristic of leaf

Trichomes are simple and stellate (branched) type. Uniseriate, unicellular and multicellular covering trichomes with pointed ends are found. The trichomes are thick walled and lignified. Paracytic type of stomata is found. Prismatic calcium oxalate crystals and are present frequently. Starch grains are not found frequently. These occur in few groups and are spherical shape. The diameter of starch grains varies from 15µ-45µ.

Powder characteristic of stem

Fragment of cork consists of rectangular or squarish parenchymatous cell. Cortex is made up of polygonal parenchymatous cells. Few parenchymatous cells contain starch grains. Both spiral and bordered pitted vessels are found.Both lignified and non lignified fibres are found. These are large number of thick walled elongated fibres. The length of phloem fibres varies from 105µ-525µ. Starch granules are abundant, spherical and compound. The diameter is from 22.5µ-45µ. Crystals are large in size. These are appeared in squarish or prism.

Powder characteristic of root

The cork contains thin walled rectangular parenchymatous cell. Fragment of cortex shows several layer of thin walled squarish rectangular parenchymatous cell. These are brown to dark brown in colour. Wood element consists of border pitted xylem vessels. They are well developed. Prismatic crystals are found. Starch granules are not frequently found. These are spherical, few starch grains are bi-head. The diameter varies from 22.5µ-75µ.

Determination of physico-chemical parameters

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 *Triumfetta rhomboidea* of leaf were found to be 10.33%w/w, 4%w/w, 2.2%w/w and 12.2%w/w. Total ash of *Triumfetta rhomboidea* leaf were found to be more than water soluble ash and acid insoluble ash. 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 acid insoluble. The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Triumfetta rhomboidea* of stem were found to be 7%w/w, 3%w/w, 1.5%w/w and 11.5%. The total ash and water soluble ash value of *Triumfetta rhomboidea* stem powder were found to be more. Sulphated ash was found to be more than total ash and water soluble ash. Acid insoluble ash was very less than total ash, water soluble ash and sulphated ash. The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Triumfetta rhomboidea* root were found to be 7%w/w, 4.5%w/w, 4%w/w and 11%w/w. Toatal ash was more than

Table 3: Behavior of powdered leaf, stem and root of *Triumfetta rhomboidea* with chemical reagent

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

Table 4: Fluorescence analysis of powder of *Triumfetta rhomboidea*

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

ater soluble and acid insoluble ash. Sulphatated ash was more than total ash water soluble ash and acid insoluble ash. Acid insoluble ash was very less than other ash values.

Total extractive values

The extractive values were determined to find out the amount of soluble compounds. The chloroform, acetone, methanol and water extractive values of leaf of *Triumfetta rhomboidea* were 3.2%w/w, 5.6%w/w, 19.2%w/w and 32%w/w. The leaf shows more amount of water soluble compound than chloroform, acetone and methanol extract. The chloroform, acetone, methanol and water extractive values of stem of *Triumfetta rhomboidea* were 3.2%w/w,0.4%w/w, 1.4%w/w and 5%w/w. The stem showed more amount of water and chloroform soluble component than acetone and methanol extract. The chloroform, acetone, methanol and water extractive values of root of *Triumfetta rhomboidea* were 1%w/w, 0.66%w/w, 2.6%w/w and 6%w/w. The root showed more amount of water and methanol soluble components than chloroform and acetone extract.

Loss on drying

The moisture content of leaf, stem and root were found to be 9.5%w/w, 10%w/w and 7.5%w/w. Stem has more moisture content than leaf and root.

Behavior of powdered materials towards chemical reagent

The behavior of the powdered leaf, stem and root were treated with picric acid, con. Sulphuric acid, con. Hydrochloric acid, con. Nitric acid, glacial acetic acid, 5%

ferric chloride, sodium hydroxide (5N),potassium hydroxide (5N), iodine/20 solution were observed.

Fluorescence analysis of powder of Triumfetta rhomboidea

Fluorescence analysis of entire leaf, stem and root has been carried out in day light and under UV light. The powders were treated with differing organic solvents and solutions and observed in normal day light and under UV light.

DISCUSSION

In view of the commercialization of formulations of traditional plants and for the development of new chemotherapeutic agents, quality control of medicinal plants used in traditional medicine is becoming more important. Adulterated and substituted medicinal plants may produce severe health related problems when taken by the patients and may cause legal problems in the pharmaceutical industries[17].

The botanical identification and physico-chemical characters may be helpful for pharmacognostical study and standardization of herbal drugs. It may be considered as the diagnostic tool for the researchers who are involved in the evaluation of herbal drugs from indigenous source.

The microscopical study of medicinal plants is a major tool for the authentification of drugs especially for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost[18].

The moisture content of leaf, stem and root were found to be 9.5%w/w, 10%w/w and 7.5%w/w. The less value of moisture content of drugs could prevent content bacterial, fungal or yeast growth through storage[19].

The total ash, water soluble ash, acid insoluble ash and sulphated ash of *Triumfetta rhomboidea* of leaf were found to be 10.33%w/w, 4%w/w, 2.2%w/w and 12.2%w/w respectivey. Ash values used to find out quality, authenticity and purity of unsophisticated drug and also these values are important quantitative standards[20].

The extractive values of the drug are valuable to estimate the chemical constituents present in the drug and also help to evaluate certain phytoconstituents soluble in a particular solvent[21].

Phytochemical screening gives the information of nature of chemical constituents of the drug. Preliminary phytochemical screening revealed the presence of carbohydrate glycosides, phytosterol, steroids, flavonoids, tannin & phenolic compounds and triterpenoids in this plant. The crude drugs do not produce fluorescence in daylight, but they can produce fluorescence, when observed in ultra violet light. The powder crude drug does not produce fluorescence on their own, but they may be converted into fluorescent derivatives in presence of different solvents and reagents. The fluorescent method is helpful in determination of the drug sample over a satisfactory concentration range without several time consuming dilution steps prior to the analysis. So, this fluorescence study could be one of the parameter for evaluation of crude drugs[19].

CONCLUSION

This research paper discusses not only pharmacognostical and phytochemical characteris but also microscopic and fluorescence characters of the different parts of the plant. These characteristics can be used further as identification and authentication parameters of the plant. The data could be useful to differentiate closely related plant species having similar phytoconstituents and pharmacological activities.

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