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Research Article

PRELIMINARY TESTS AND ANATOMICAL FEATURES OF FEW MEDICINAL WEEDS

***Veerabhadra Swamy AL[1], Amaravathi DS[2] and Kavana JM[2]**
**Post Graduate Department of Botany, J.S.S. College of Arts, Commerce and Science, B.N. Road, Mysuru – 570 025, Karnataka, India*
[2] Post Graduate Students, Post Graduate Department of Botany, J.S.S. College of Arts, Commerce and Science, B.N. Road, Mysuru – 570 025, Karnataka, India
**Author for Correspondence: vsbotany@gmail.com*

ABSTRACT
Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and are always safe. The advantage of natural drugs is their easy availability, economic and less or no side effects they have been used in traditional medicine practices. In the present investigation some medicinal weeds were analyzed, it includes *Centella asiatica, Phyllanthus niruri, Argemone mexicana, Amaranthus viridis, Asclepias currasavica, Cyperus iria* and *Portulaca oleraceae*. All the eight medicinal weeds collected during field visits were subjected to study organoleptic features, macroscopic studies, powder analysis, physicochemical limits and fluorescence analysis, single and double staining techniques.

Keywords: *Medicinal, Weeds, Double staining, Anatomy*

INTRODUCTION
There is some connection present in between man and his research for drugs in nature from the far past. There is much evidence present for most of drugs originated by plants in the way of written documents and original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illness due to which man learned to continue drugs in the bark, seeds, fruit bodies and other parts of the plants (Petrovska, 2012). Now-a-days there is a renewed interest in drugs of natural origin simply because they are considered as green medicine and green medicine is considered always safe. The advantage of natural drugs is their easy availability, economic and less or no side effects they have been used in traditional medicine practices since prehistoric times, numerous phytochemicals with potential or established biological activity have been identified. Like these, we used some weeds for the analysis of drug properties and conduct some preliminary tests like physicochemical analysis, morphological-anatomical, etc. Some important medicinal studied in the present investigations were carried out, they are *Centella asiatica, Phyllanthus niruri, Argemone mexicana, Amaranthus viridis, Asclepias currasavica, Cyperus iria, Atylosia scarbaoidesis* and *Portulaca oleraceae.* Among the eight medicinal weeds four were collected from Mysore district (*Centella asiatica, Phyllanthus niruri, Argemone Mexicana* and *Amaranthus viridis*) and remaining four were collected from Hassan district (*Asclepias currasavica, Cyperus iria, Atylosia scarbaoidesis* and *Portulaca oleraceae*).

Centella asiatica (L.) Urb., a small edible, herbaceous medicinal plant belonging to Apiaceae family native to India, Sri Lanka, Iran, New Guinea, Australia, Indonesia southern and central Africa (Arpita *et al.,* 2018). In 2006, it was ranked the third position in a priority list of most essential Indian medicinal plants based on their pharmaceutical and economic importance and also the demands for its raw material is constantly rising throughout the world. In addition to neuroprotective effect of *C. asiatica*, it has been reported to own a wide range of biological activities desired for human health such as wound healing, anti-inflammatory, antiulcer, anticonvulsant, sedative, immunostimulant, cardioprotective, anti-diabetic, cytotoxic and antitumor, antiviral, antibacterial, insecticidal, antifungal, antioxidant, and for lepra and venous deficiency treatments (Orhan, 2012). *Phyllanthus niruri* L. is a widespread tropical plant commonly found in coastal areas, known by the common names gale of the wind, stonebreaker or seed-under-leaf, belonging to the genus Phyllanthus of the family Phyllanthaceae. Its leaves and fruit are used as herbal medicine. *Phyllanthus niruri* is known for protecting the liver. It may also combat kidney stones, hence the "stonebreaker" moniker.

Argemone mexicana L. is a species of poppy found in Mexico and now widely naturalized in many parts of the world. *Amaranthus viridis* L. belongs to Amaranthaceae family and is commonly known as slender amaranth or green amaranth.

The plant *Asclepias currasavicca* L. is rich sources of glycosides, Vincetoxin, Cardenolides, hesperidin β-Sitosterol and Glucoside and now a days, standardization of herbal drugs is a topic of great concern therefore in the present study on micro morphological features, other physical values and chemical parameters on the whole of *Asclepias currasavicca* will help to identify the correct species of the plant (Mohan *et al.,* 2009). *Cyperus iria* L. is a clump-forming, annual or sometimes perennial plant with culms of grass-like leaves 8 - 80cm tall. The plant is astringent, febrifuge, stimulant, stomachic and tonic. It is used in the treatment of amenorrhoea. The whole plant is used to treat rheumatism and to regulate menstruation. The rhizomes are used as a diuretic (TPD, 2019). *Atylosia scarabaeoides* (L.) Benth, Fabaceae, locally known as Banurkali or Thitkalai, is a slender, twining herb with densely grey- dowry stems that is distributed throughout Bangladesh, India, Malaysia, China, Mauritius and Madagascar (Mohiuddin *et al.,* 2018). It has been used in folk medicine for its various medicinal properties like antimicrobial and dysenteric, anti-cholera, febrifuge and in treatment of anemia (Mohiuddin *et al.,* 2018). *Portulaca oleracea* L. is used locally for herbal medicine and as food but yet to be fully explored. The plant has been reported as a global panacea due to its several medicinal uses (Okafor and Ezejindu, 2014).

MATERIALS AND METHODS

Study location:

Plant materials for the present work were collected from Mysore and Hassan District. The latitude of Mysore, Karnataka, India is 12.311827, and the longitude is 76.652985,with the GPS coordinates of 12° 18' 42.5772" N and 76° 39' 10.7460" E. The latitude of Hassan is 13.009711, and the longitude is 76.102898, with the GPS coordinates of 13^0 0' 34.9596'' N and 76° 6' 10.4328" E.

Field survey for collection of plant materials:

Collect the proper number of samples and take them directly to the testing laboratory. Field survey was carried out in January – 2019 in and around the Mysore and Hassan District. During field visit generally, we concentrate medicinal weeds for the study of preliminary analysis and anatomical features. The plants used in the present investigation were selected based on their medicinal use and weed (interesting features) properties.

Organoleptic character:

Organoleptic evaluation can be done by sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure the quality of a particular drug. Organoleptic characters such as shape, size, colour, Odour, taste and fracture of stem bark, leaf structure like margin, apex, the base surface, venation, and inflorescence, etc. are evaluated.

Macroscopic study:

The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens of different plant weeds.

Microscopic study:

Microscopy is used to determine the structural, cellular and internal tissue features of botanicals. It is usually used to identify and differentiate two herbals that are similar. This is the commonly used technique, convenient, quick and can be applied to proprietary medicines too. Microscopic inspection alone can't always provide complete identification but when used in the association with other analytical methods that is by taking plant section and qualitative microscopic test for detection of cellular contents in all the different plant weeds. Plant part under study took in the form of the appropriate (transverse / cross) section to study the presence or absence of type (shape) of cells or tissues. Some of the chemicals like Phloroglucinol, chloral hydrate, safranin, methyl orange, etc., use for clear visualization of cellular content. Using microscope detecting various cellular tissues, trichomes, stomata, starch granules, calcium oxalate crystals, and aleuronic grains are some of the important parameters which play an important role in the identification of crude drug. The crude drug can also be identified microscopically by cutting the thin TS, especially by staining them with proper staining reagents.

Powder analysis:
Leaves are air dried and powdered. The fine powder was used for microscopic characterization and also for macroscopic analysis. Drug powder was treated with different reagents and the colour change was noticed under day light. Dried powder took for studying the presence or absence of cellular contents (type/ shape) by using a microscope. The microscopic evaluation also includes the study of constituents in the powdered drug by the use of chemical reagents. These reagents used due to the abundance of cellular contents, the presence of coloring matters, shrinkage or collapse of cell wall which creates hurdles in microscopic evaluation of different plant weeds.

Physicochemical parameters:
Parameters studied include pH value (1% solution), extractive values (water soluble, alcohol soluble) and ash values (total ash, acid insoluble ash, water-soluble ash) of all the different plant weeds will be calculated.

Fluorescence analysis:
The shade dried plant leaves were powdered in mechanical grinder 5 grams of leave powder was weighed; 37.5 ml of solvent was added and kept for three days. The extract was filtered using Whatman No. 1 filter paper and the supernatant was collected. The residue was again extracted two times (with three days of the interval for each extraction) and supernatants were collected. The supernatant was pooled and evaporated at room temperature until the volume was reduced to 150 ml. Finally, the extract of the leaves powder with ethanol was prepared and stored in airtight bottles for subsequent analysis. The extract was centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper. The sample was diluted to 1:10 with the same solvent. The extracted was scanned at a wavelength ranging from 400 to 700 nm using a spectrophotometer and that values were recorded.

Single staining method:
Take fresh plant material (petiole, stem) and they preserved in fixative solution FAA (formalin 5ml + acetic acid 5ml + 70% ethyl alcohol 90ml) for more than 48 hrs. The preserved specimens were cut into the thin transverse section and were stained with safranin. The selected diagnostic characters of the transverse section and were photographed under suitable magnification (4X, 10X, 40X and 100X) of the microscopic field.

Double staining method (Safranin-Fast Green):
Keep the material to be stained in safranin for three to five minutes and then wash it with water. See under the microscope that only thick-walled cells are stained. Excess of stain is de-stained by acid alcohol. Again wash the material very thoroughly with water so that even the traces of acid are removed. Now stain the material with few drops of fast green for a few seconds. Time for keeping the material in fast green varies from a few seconds to one minute for different materials. Wash the material with glycerin and mount in a drop of glycerin. The technique of Double staining method was first time used for the anatomical studies of all the weeds.

RESULTS AND DISCUSSION

Field survey was carried out in January – 2019 (Fig. 1) in and around the Mysore District and the data of field visit presented in Table – 1. During field visits few medicinal weeds are brought to the laboratory in the polythene bag. The plants used in the present investigation were selected based on their medicinal use and weed (interesting features) properties. Further, medicinal weeds are subjected to further studies i.e., study of organoleptic features, macroscopic studies, powder analysis, physicochemical parameters, fluorescence analysis, single staining method and double staining techniques.

Organoleptic features: Organoleptic studies of all the eight species were carried out in the present investigation (Table – 2). *Centella asiactica* shows dark green in colour with Acrid, bitter and sweet taste. *Phyllanthus niruri* shows pale bluish green in colour with an unpleasant smell and bitter taste, Similarly, *Argemone mexicana* and *Amaranthus viridi s*show green in color with an unpleasant smell and bitter taste. Except for *Centella asiactica* remaining three species (*Phyllanthus niruri*, *Argemone mexicana* and *Amaranthus viridis*) are terrestrial in nature but *Centella asiactica* exhibit cool nature. On the other hand, *Asclepias curassavicca* shows green in colour with bitter taste. *Cyperus iria* shows

Table 1: Field visit data for the collection of medicinal weeds

Sl. No.	Plant Name	Date of Collection	Time of Collection	Place of Collection	Part used for the study
1	*Centella asiatica*	18/ 01/ 2019	10:30 AM	B.N. Road, Mysuru	Leaves and Petioles
2	*Phyllanthus niruri*	17/ 01/ 2019	4:37 PM	Nanjanagudu Town, Mysore District	Stem
3	*Argemone mexicana*	17/ 01/ 2019	4:30 PM	Nanjanagudu Town, Mysore District	Leaves and Stem
4	*Amaranthus viridis*	17/ 01/ 2019	4:37 PM	Nanjanagudu Town, Mysore District	Stem
5	*Asclepias currasacicca*	18/1/2019	9:13 AM	Shankaripuram, Hassan.	Stem
6	*Atylosia scarboidesis*	17/2/2019	8:00 AM	Baldare, Chanrayapatna	Stem
7	*Cyperus iria*	21/1/2019	1:28 PM	Bellikoppalu, Hassan.	Stem
8	*Portulaca oleraceae*	21/1/2019	2:16 PM	Bellikoppalu, Hassan.	Leaves and Stem

Figure 1: Morphology of *Centella asiatica* (A and B), *Phyllanthus niruri* (C), *Argemone mexicana* (D), *Amaranthus viridis* (E), *Asclepias currasavicca* (F), *Cyperus iria* (G), *Atylosia scarabaeoides* (H) and *Portulaca oleracea* (I).

Table 2: Organoleptic features of *Centella asiactica, Phyllanthus niruri, Argemone mexicana,* and *Amaranthus viridis, Asclepias currasavicca, Atylosia scarboidesis, Cyperus iria* and *Portulaca oleracea*

Sl. No.	Plant name	Colour	Odour	Taste	Nature
1	*Centella asiactica*	Dark green	Characteristic	Acrid, bitter, sweet	Cool natured
2	*Phyllanthus niruri*	Pale bluish-green	Unpleasant-smelling	Bitter	Terrestrial nature
3	*Argemone mexicana*	Green	Unpleasant-smelling yellow sap when cut	Bitter	Terrestrial nature
4	*Amaranthus viridis*	Green	Unpleasant-smelling	Bitter	Terrestrial nature
5	*Asclepias currasavicca*	Green	No characteristic	Bitter	Smooth
6	*Atylosia scarboidesis*	Green	Aromatic	Acrid	Fibrous
7	*Cyperus iria*	Pale yellowish green	No characteristic	Slightly bitter and astringent	Smooth
8	*Portulaca oleracea*	Green with red	Pungent	Slightly sour	Smooth

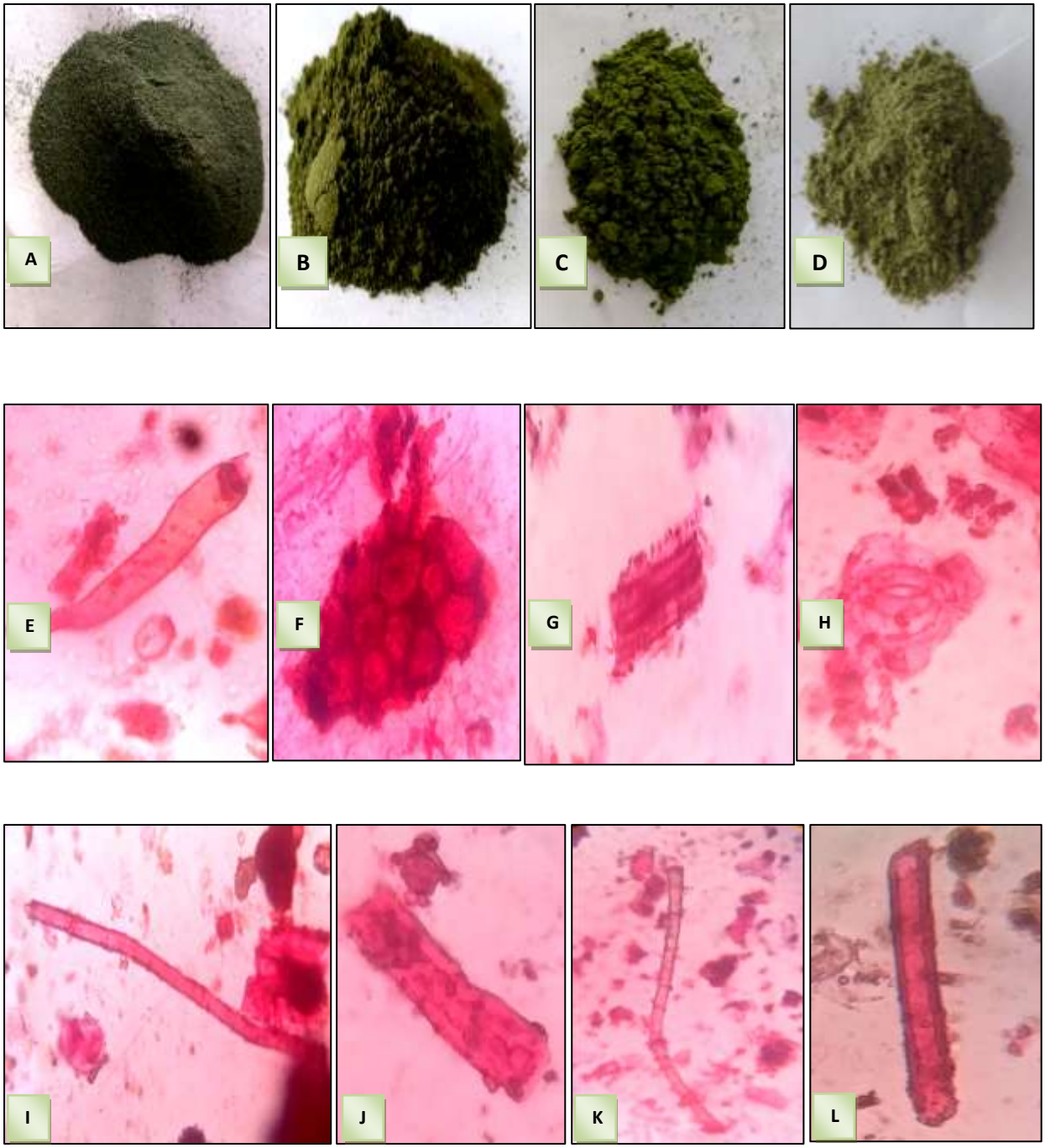

Figure 2: A-D. Powder analysis of *Amaranthus viridis, Argemone mexicana, Centella asiatica* and *Phyllanthus niruri,* E. Phloem fiber, F. Cork cells, G. Xylem vessel with spiral thickening, H. Stomata, I. Simple fiber, J. Simple pitted vessels, K. Woody fiber, L. Pitted vessel.

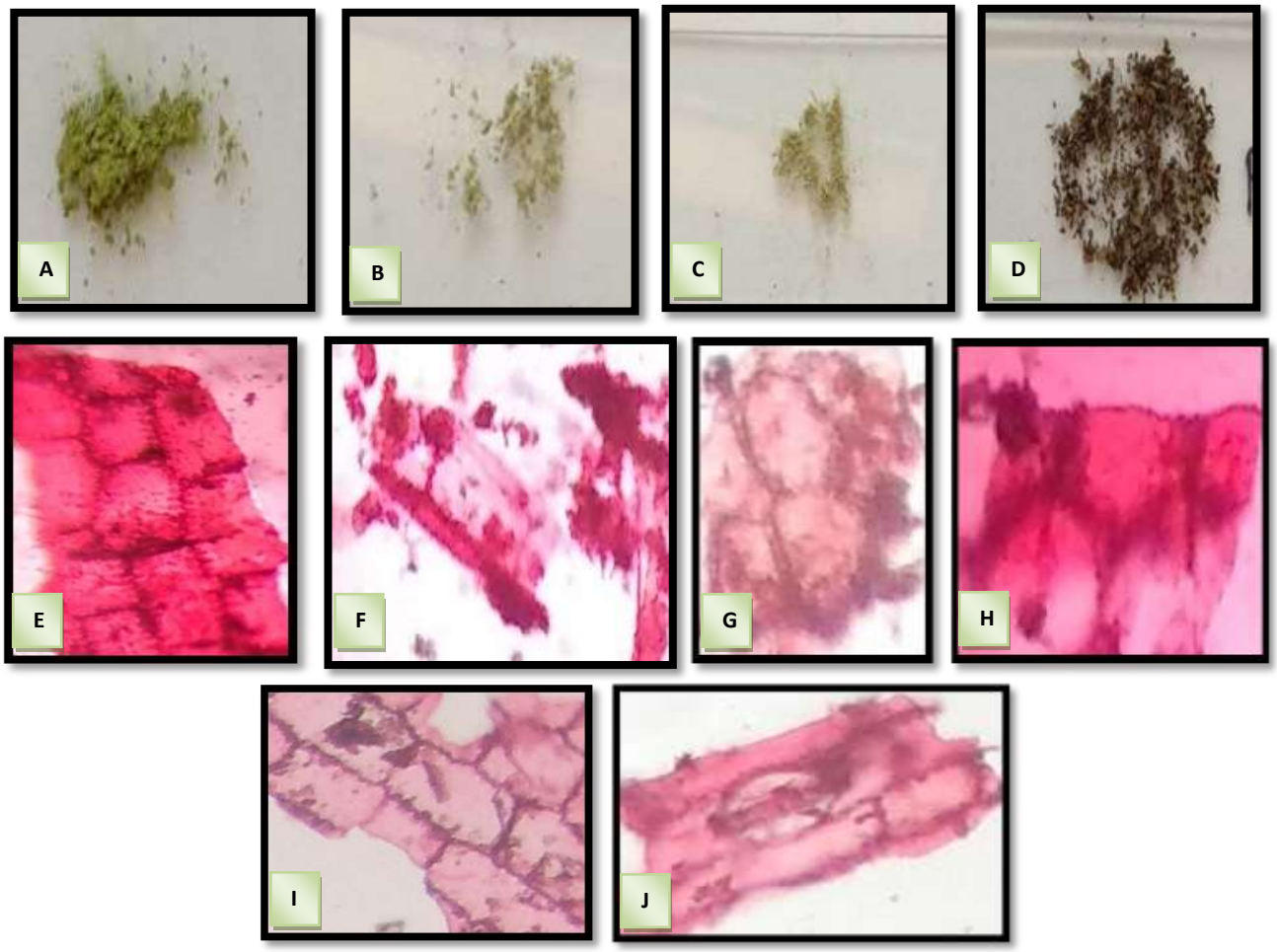

Figure 3: A-D. Powder analysis of *Asclepias curassavicca, Atylosia scarboidesis, Cyperus iria* and *Portulaca oleraceae,* E. Parenchyma cells seen in *Asclepias curassavica*, F. Phloem fibres, G. Chlorenchyma cells, H. Collenchymas cells, I. Parenchyma cells seen in *Cyperus iria*, J. Stomata seen in *Cyperus iria*.

Table 3: Physicochemical parameters of *Centella asiactica, Phyllanthus niruri, Argemone mexicana,* and *Amaranthus viridis. Asclepias currasavicca, Atylosia scaraboidesis, Cyperus iria,* and *Portulaca oleracea*

Parameters		***Centella asiatica***	***Phyllanthus niruri***	***Argemone mexicana***	***Amaranthus viridis***	***Asclepias currasavicca***	***Atylosia scarboidesis***	***Cyperus iria***	***Portulaca oleracea***
pH value	1% pH solution	6.85	7.08	6.9	7.10	6.96	6.66	6.56	6.29
Extractive values	Water soluble extractive values	2.16%	2.88%	17.6%	7.92%	20.8%	0.8%	47.6%	7.6%
	Alcohol soluble extractive values	3.56%	1.5%	13.6%	6.04%	11.6%	45.6%	0.76%	7.2%
Ash values	Total ash	35%	40.5%	21%	40%	15.2%	95%	5.28%	48%
	Acid insoluble ash	10.2%	4.08%	4.55%	9%	1%	1.4%	19.4%	5%
	Water soluble ash	6.2%	6.12%	5.2%	7.6%	16%	1.85%	5.2%	15%

Table 4: Fluorescence analysis by spectrophotometer of *Centella asiactica, Phyllanthus niruri, Argemone mexicana,* and *Amaranthus viridi, Asclepias currasavicca, Atylosia scarboidesis, Cyperus iria, Portulaca oleraceae*

Wave length	*Centella asiatica*	*Phyllanthus niruri*	*Argemone mexicana*	*Amaranthus viridis*	*Asclepias currasavicca*	*Atylosia scarboidesis*	*Cyperus iria*	*Portulaca oleracea*
400 nm	0.41	0.27	0.49	0.47	0.76	0.74	0.70	0.64
420 nm	0.98	0.71	1.05	1.03	0.83	0.81	0.77	0.71
470 nm	1.10	0.54	1.24	1.23	0.97	0.70	0.86	0.68
500 nm	1.43	0.45	1.52	1.49	1.63	1.17	1.54	1.16
530 nm	1.03	0.36	1.10	1.08	1.19	0.89	1.10	0.85
620 nm	1.17	0.36	1.30	1.28	1.12	0.48	0.97	0.57
660 nm	0.99	0.38	1.13	1.12	0.99	0.50	0.87	0.61
700 nm	0.17	0.09	0.26	0.24	0.57	0.52	0.51	0.44

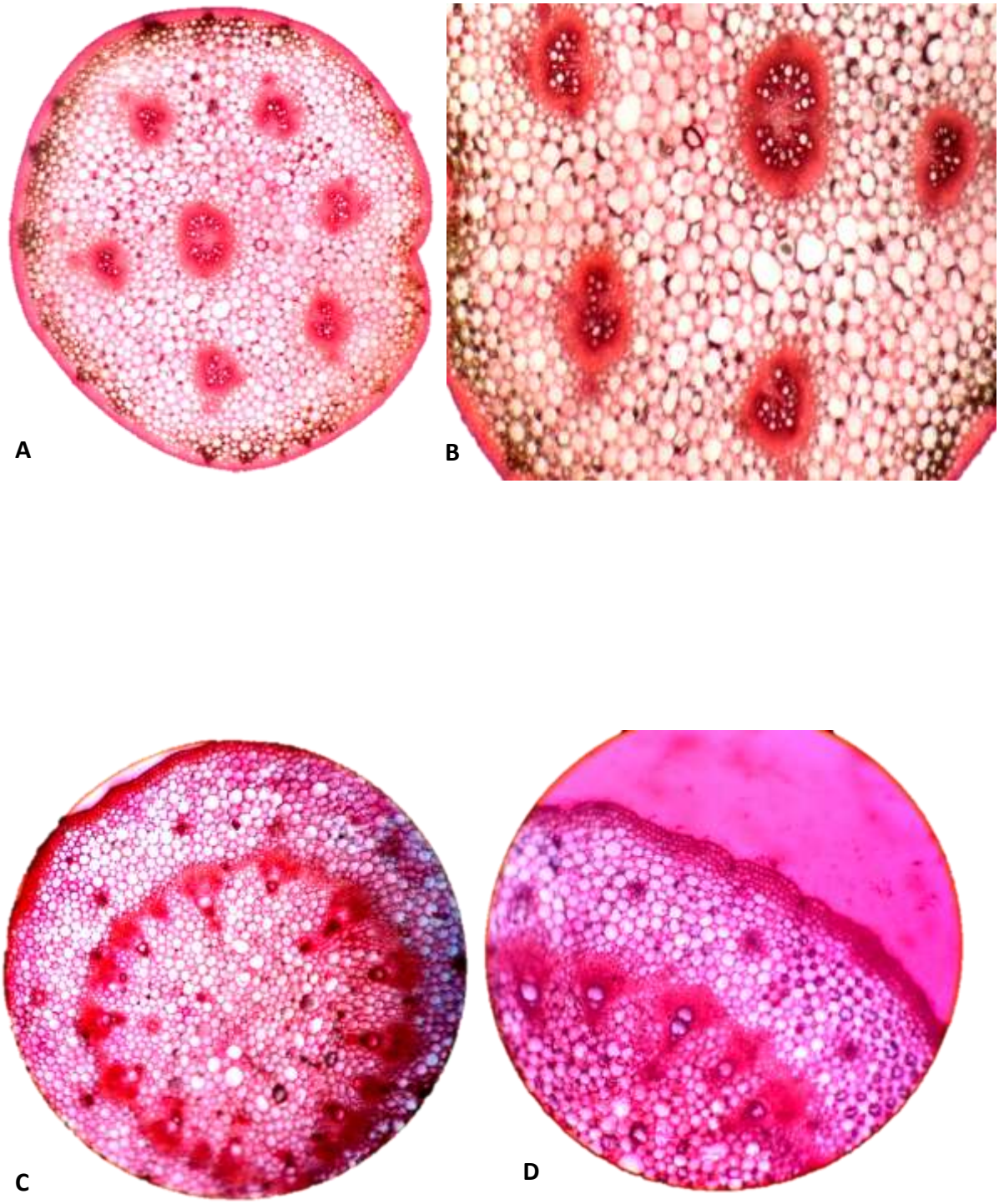

Figure 4: A. T. S. of petiole of *Centella asiatica*, B. Section shows epidermis, hypodermis, cortex region and distealic vascular bundle, C. T.S. of Stolon of *Centella asiatic a*D. Stolon shows cortex, pith, vascular bundles.

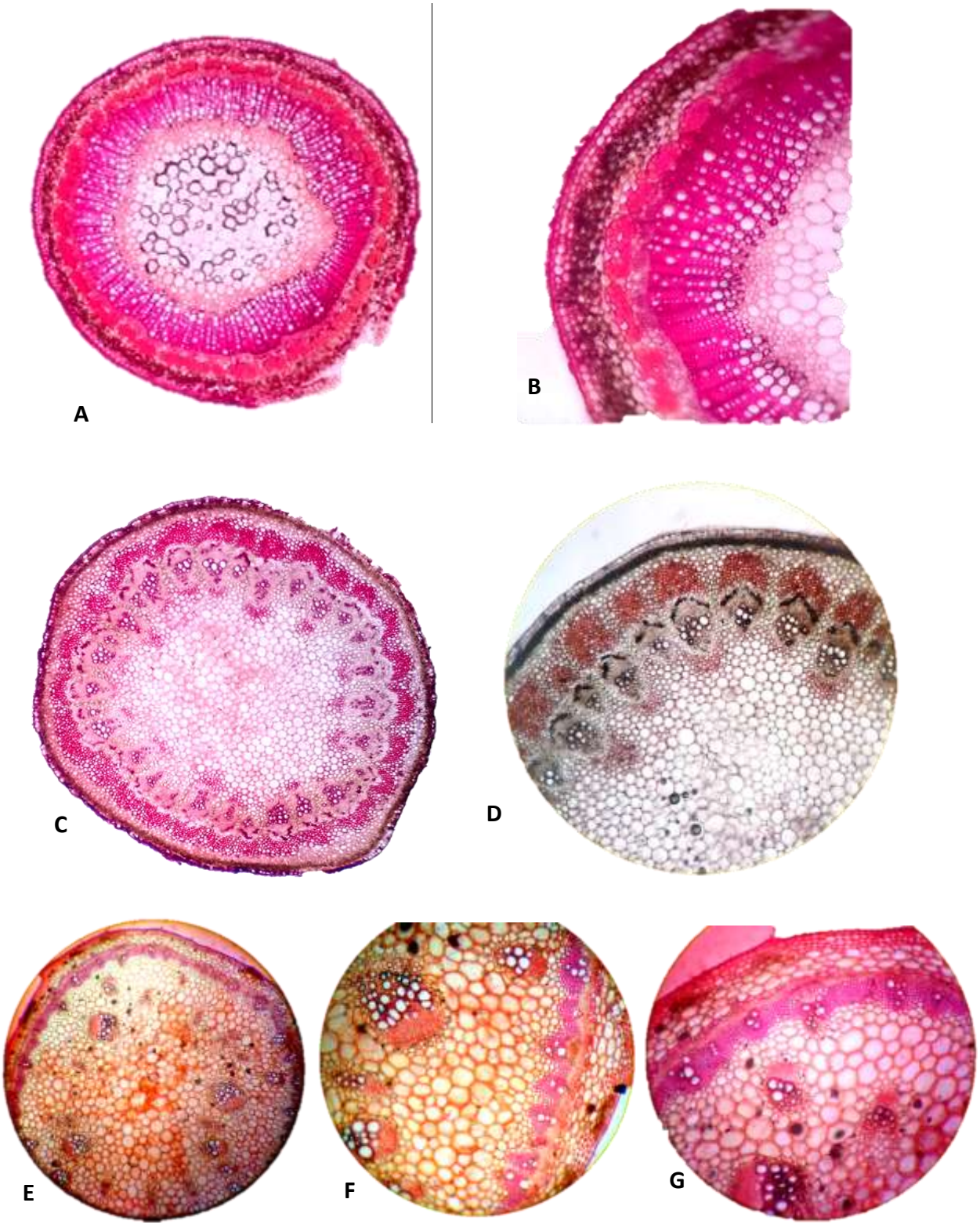

Figure 5: A. TS of stem of *Phyllanthus niruri*, B. Section shows epidermis, hypodermis, vascular bundles, pith region. C. TS of *Argemone mexicana* section, D. Section shows epidermis, hypodermis, vascular bundles present in cortex region. E. TS of stem of *Amaranthus viridis* section, F. Section shows vascular bundles in cortex region G. Section shows epidermis, hypodermis.

Figure 6: TS of stem of *Asclepias currasavicca* (A), TS of stem of *Atylosia scaraboidesis* (B), TS of *Cyperus iria* (C and D), TS of *Portulaca oleraceae* (E)

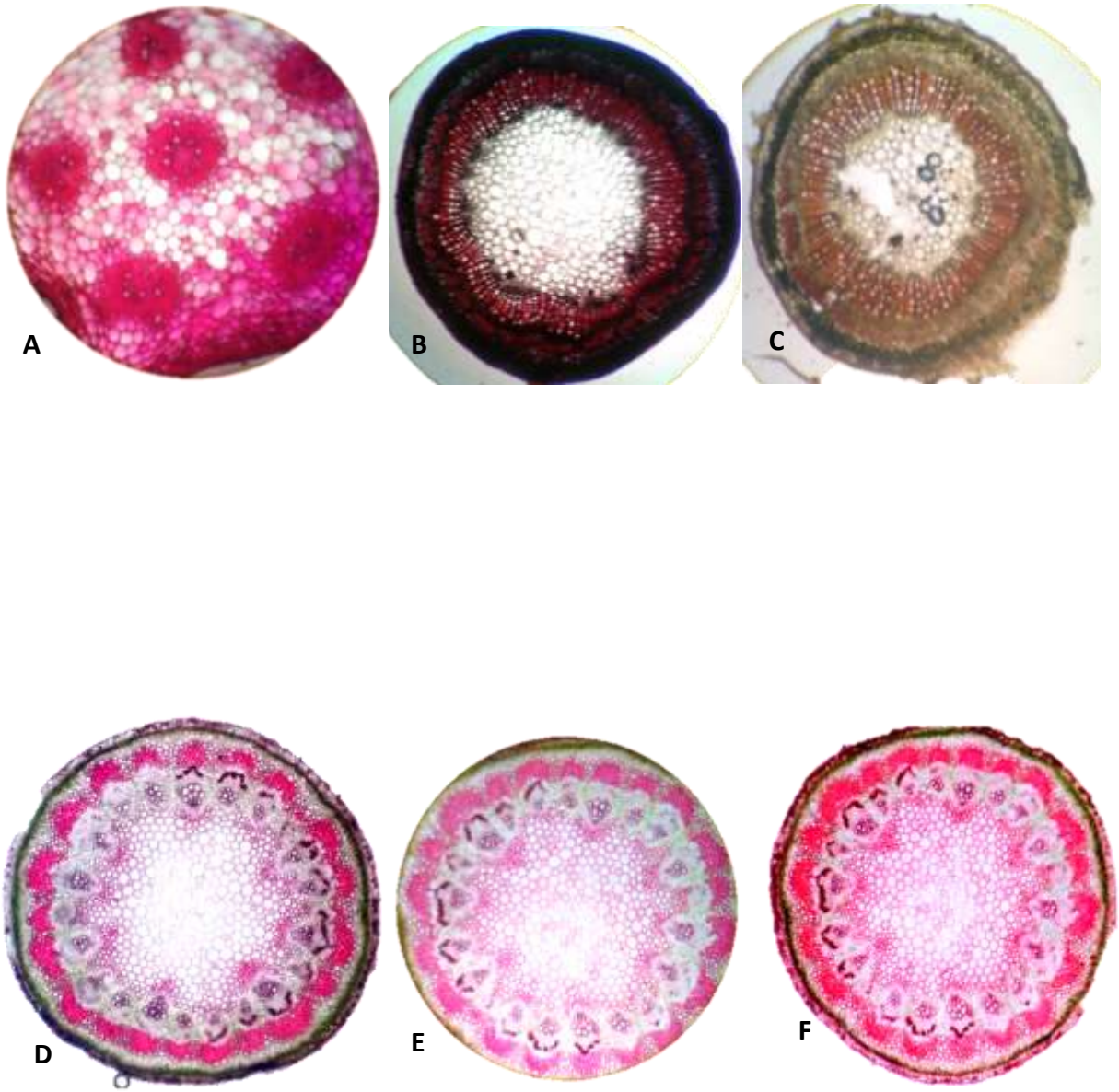

Figure 7: A. Double staining of TS of the stem of *Centella asiatica*, B. Double staining of TS of the stem of *Phyllanthus niruri*, C. Double staining of TS of the stem of *Amaranthus viridis*, D-F. Double staining of TS of the stem of *Argemone mexicana*

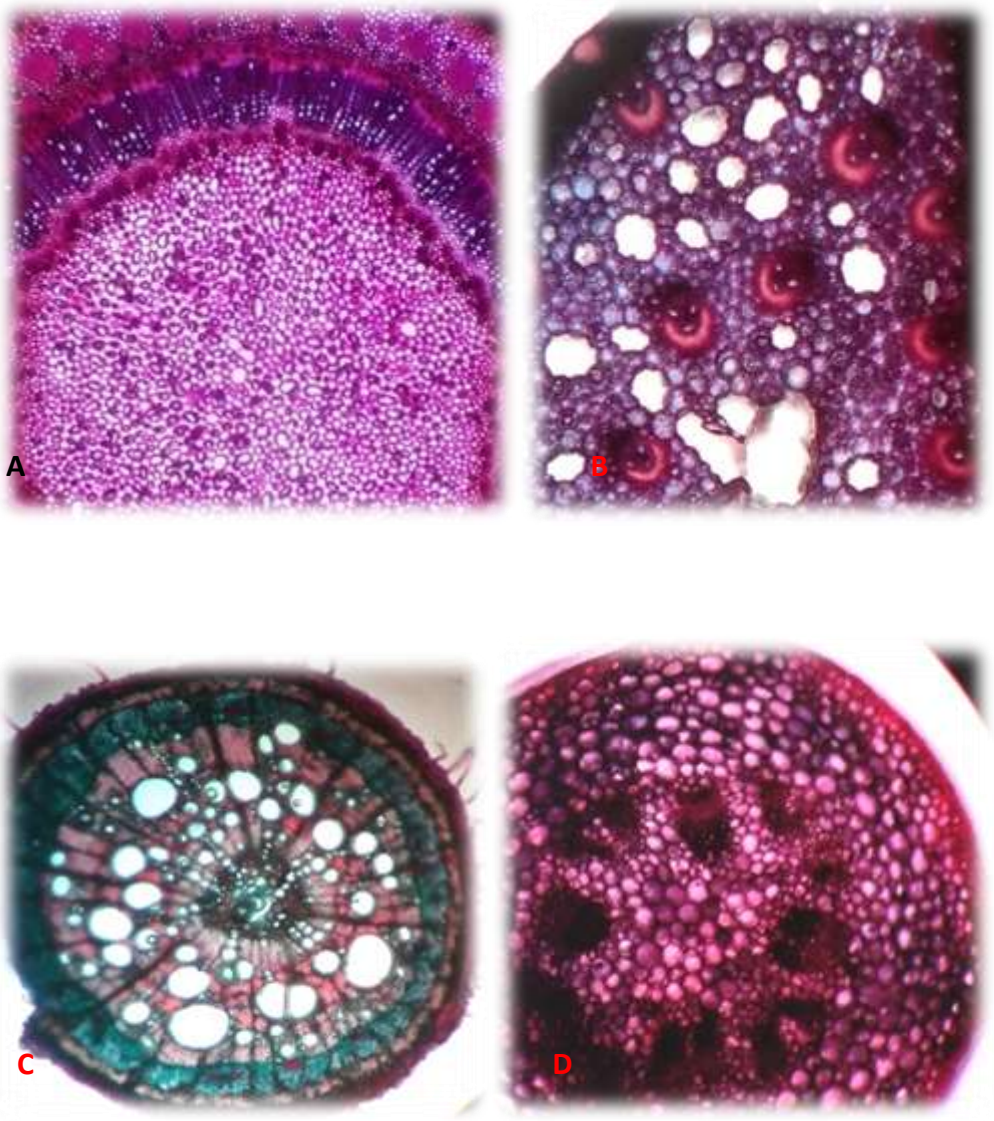

Figure 8: Double staining of TS of the stem of *Asclepias curassavicca* (A), *Cyperus iria* (B), *Atylosia scaraboidesis* (C) and *Portulaca oleraceae* (D)

pale yellowish green in colour with slightly bitter and astringent taste and no characteristic odour, similarly *Atylosia scarboides* is show green in colour with an aromatic odour and acrid taste with fibrous nature. Except *Cyperus iria,* remaining three species (*Atylosia scarboidesis, Asclepias curassavicca, Portulaca oleraceae*) may grow in warm climate but *Cyperus iria* needs wetland characteristic and except *Portulaca oleraceae* all three species are non-succulent.

Leonti *et al.*, in 2002, provides evidence for a highly significant association between organoleptic properties of plants and the use of Popolucaas medicine. Similarly, Arya and Thakur in 2012 conduct organoleptic and microscopic analysis of *Gentiana regeliana* and examination revealed various diagnostic characters of stem, flower and leaves. Singh *et al.*, in 2017 carried out organoleptic characters of aqueous extracts of *Trigonella foenum, Allium sativum, Aloe vera* and *Phyllanthus niruri.*

Macroscopic studies:

The morphological studies of eight species of medicinal weeds were studied using "Flora of the presidency of Madras" published by Botanical Survey of India as a standard reference.

Centella asiatica

It is a perennial herb in the flowering plant belongs to the Apiaceae family. The stems are slender, creeping, stolons connecting the plant to each other. Leaves are arranged in small clusters of 1 to 5. Blades are attached by long petiole with 1 to 50 cm long. The leaflet is gutter and base sheathing, papery, pubescent or woolly on the lower surface, especially the ribs but uppersurface glabrous in nature. The leaf margin is crenate to dentate. Venation is palmate. The inflorescence is very contracted with 1 to 5 flowers in an umbel, in the axils of leaves. Flowers are small greenish white. Corolla with 5 petals, oval to triangular, 5 stamens alternate with the petals. The position of the ovary is inferior with two separate carpels, style, and stigma. Flowers are bisexual, sessile or with pedicels slender with 15 mm long. Peduncle erect, long 1.5 to 5 cm, shorter than the petioles. The rootstock consists of rhizomes, growing vertically down. They are creamish in color and covered with root hairs.

Phyllanthus niruri

This plant belongs to phyllanthaceae and it is an erect annual herb, slender, branched. The stem is slightly woody greenish in colour and branched at the base and angular. The leaves are numerous, small, green, subsessile, closely arranged, oblong-shaped, obtuse, having short petiole and they arranged alternately on each side of the stem. Fruit is very small, and it present under the leaves. The stem is slightly glabrous. Root is branched with a tap root system.

Argemone mexicana

Argemone mexicana is an annual herb with a slightly branched taproot. Its stem is branched and usually extremely prickly. It exudes a yellow juice when cut. It has showy yellow flowers. Leaves are thistle-like and alternate, without leaf stalks (petioles), toothed (serrated) and the margins are spiny. The grey-white veins stand out against the bluish-green upper leaf surface. Flowers are at the tips of the branches (are terminal) and solitary, yellow. Fruit is a prickly oblong or egg-shaped (ovoid) capsule. Seeds are very numerous, nearly spherical, covered in a fine network of veins, brownish black. Taproot system with slightly branched.

Amaranthus viridis

This plant belongs to Amaranthaceae family and it is an annual herb erect. The stem is slender, branched, angular, and glabrous. Leaves are light green and deeply veined and it has a long leaf stalk and has broad base tapering to a pointed base. The inflorescence is a dense spike, often with many branches. Flowers are green, axillary or terminal, often paniculate spikes, Bracts, and bracteoles. Fruit capsules are wrinkled, indehiscent (not opening to release seed when ripe), small and brown. The fruits contain smooth and glossy seeds. Root is a branched tap root system.

Asclepias curassavicca

It is an erect, evergreen perennial subshrub that grows to 1m in height. It has a woody base, stems with milky sap, and dark green lance- shaped leaves 5-15 cm long. It produces showy orange and red flowers in umbels with brilliant red centres. It blooms almost continuously which has made it a favourite as a cultivated plant in gardens and attracting bees, hummingbirds and butterflies to the

garden. The fruits are spindle shaped 3-4 inch pods which eventually split open to release little flat seeds that drift away on silky strands.

Cyperus iria

It is a tufted annual herb, or occasionally perennial, with fibrous roots, 15-75 yellowish red roots; 10-70 cm tall. The stem is sharply 3 angled, tufted, smooth, 5-80 cm high. The leaf is basal, rough to touch in upper part, linear, flaccid, with gradually tapering point and 3-8 mm wide; sheath reddish or purplish brown, enveloping the stem at base. The inflorescence is simple or compound umbel composed of numerous erect-spreading 3-10 mm long flattened spikelets. The fruit is three angled, 1.0 – 1.5 mm nut with slightly concave sides, and shiny dark brown to black.

Atylosia scarboidesis

It may be annual or a perennial, making it a flexible crop for subsistence farmers. The branches of *A. scarabaeoides* can be straight or winding and upto 135 cm in length. It has pinnate leaves, typically arranged in a trifoliate manner with flowers that are yellow with red veins. The pods of *A. scarabaeoides* are oblong in shape, typically 11-34 mm in length and 6-10 mm in width. The seed pods are densely covered in a combination of short and long hairs and are typically a dark purple colour, containing anywhere from 1-7 seeds. The seeds of *A. scarbaeoides* range from 2.4 -4 mm long, 1.8-3 mm wide, and 1-2 mm thick and are either black in colour or speckled.

Portulaca oleraceae:

It is an annual succulent, 10-40 cm tall that belongs to the Portulacaceae family. The stems can either grow horizontally or vertically. The dark green egg-shaped leaves are arranged opposite or alternate. The plant blooms from July to September and the small yellow flowers wither early. The fruit is 4-9 mm large, oval shaped capsule containing numerous small, shiny black seeds. Garden purslane is somewhat taller (upto 60 cm) with erect stems. Both the stems and leaves are very fleshy.

Powder analysis:

Powder analysis of *Amaranthus viridis*, *Argemone mexicana*, *Centella asiatica*, and *Phyllanthus niruri* reveals in Fig. 2 from A-L. It shows the presence of Phloem fiber, Cork cells, Xylem vessel with spiral thickening, Stomata, Simple fiber, Simple pitted vessels, Woody fiber and Pitted vessel were recorded in all the four species of medicinal weeds. This examination was done under the microscope after the preparation of powder from shade dried plant materials. Powder analysis of *Asclepias curassavicca, Atylosia scarboidesis, Cyperus iria, Portulaca oleraceae,* reveals in Fig. 3 from A-J. It shows the presence of parenchyma cells, Collenchyma cells, stomata cells with stomata, simple fiber, were recorded in all the species of medicinal weeds. This examination was done under the microscope after the preparation of powder from shade dried plant materials.

Physicochemical parameters:

When we subjected to analysis of physicochemical parameters of all the medicinal weeds, parameters studied include pH value (1% solution), extractive values (water soluble, alcohol soluble) and ash values (total ash, acid insoluble ash, water-soluble ash) were calculated. All the four species collected from Mysore districts shows moderate pH value i.e., 6.85, 7.08, 6.9 and 7.10 was recorded in *Centella asiactica, Phyllanthus niruri, Argemone mexicana,* and *Amaranthus viridis* respectively (Table – 3). Highest amount of water-soluble extractive values recorded in *Argemone mexicana* (17.6%) followed by *Amaranthus viridis* (7.92%), *Phyllanthus niruri* (2.88%) and *Centellaasiactica* (2.16%) and highest amount of alcohol-soluble extractive values were recorded in *Argemone mexicana* (13.6%) followed by *Amaranthus viridis* (6.04%), *Centella asiactica* (3.56%) and *Phyllanthus niruri* (1.5%). Remaining all four species among eight shows moderate pH value i.e., 6.96, 6.66, 6.56, and 6.29 was recorded in *Asclepias curassavica, Atylosia scarboidesis, Cyperus iria, Portulaca oleracea,* respectively (Table – 3). It is collected from Hassan districts. Highest amount of water-soluble extractive values recorded in *Cyperus iria* (47.6%) followed by *Asclepias curassavicca* (20.8%), *Portulaca oleraceae* (7.6%) and *Atylosia scarboidesis* (0.8%) and highest amount of alcohol-soluble extractive values were recorded in *Atylosia scarboidesis* (45.6%) followed by *Asclepias curassavicca* (11.6%), *Portulaca oleraceae* (7.2%) and *Cyperus iria* (0.76%).

In case of ash value, among the four species collected from Mysore districts highest total ash recorded in *Phyllanthus niruri* (40.05%), followed by *Amaranthus viridis* (40%), *Centella asiactica* (35%), and *Argemone mexicana* (21%). In the present study, least amount of acid insoluble ash

present in *Amaranthus viridis* (9%) and highest in *Centella asiactica* (10.2%). Similarly, the least amount of water soluble ash present in *Argemone mexicana* (5.2%) and highest in *Amaranthus viridis* (7.6%) (Table-3). Similarly, among the four species collected from Hassan districts highest total ash recorded in *Atylosia scarboidesis* (95%), followed by *Portulaca oleraceae* (48%), *Asclepias currasavicca* (15.2%), and *Cyperus iria* (5.28%). In the present study least amount of acid insoluble ash present in *Asclepias currasavicca* (1%) and highest in *Cyperus iria* (19.4%). Similarly, the least amount of water soluble ash present in *Atylosia scarboidesis* (1.85%) and highest in *Asclepias currasavicca* (16%) (Table-3).

Importance of pharmacognostic study of medicinal plants was studied by Sumitra in 2014. In this article discusses the need and emphasizes the importance of the pharmacognostic study of medicinal plants. In this study used some important parameters for standardization and authentication of medicinal plants with the help of which adulteration and substitution can be prevented. All the parameters to be evaluated in a pharmacognostic study such as organoleptic characters, macroscopic study, microscopic study, powder study, physicochemical analysis (moisture content, loss on drying, ash values, extractive values), phytochemical analysis, fluorescence analysis is enlisted along with their importance.

Chemical and pharmacological aspects of *Argemone mexicana* was being studied by Goutam *et. al.* in 2013. He explained the Papaveraceae, informally known as the poppy family, are an ethnopharmacologically important family of 44 genera and approximately 760 species of flowering plants. This work offers a review addressing the detailed chemistry and pharmacology of *Argemone mexicana* regarded as one of the most significant plant species in a traditional system of medicine. The plant is used in different parts of the world for the treatment of several ailments including tumors, warts, skin diseases, inflammations, rheumatism, jaundice, leprosy, microbial infections, and malaria. Interestingly, the plant is the source of a diverse kind of chemical constituents although alkaloids are most abundant. Beyond pharmaceutical efficacies, certain plant parts also show toxic effects as well.
Pharmacognostic studies in *Solanum capsicoides* all is being studied by Anusree and Anilkumarin 2018. They explained about a detailed analysis of morphological and anatomical features of *Solanum capsicoides* and it would be helpful for pharmacognostic identification. Phytochemical screening and histochemical test were performed for the confirmation and localization of the phytoconstituents present in the species. Among the morphological features, the phytochemicals identified in the plant were flavonoids, coumarins, alkaloids, tannins, steroids, saponins, phenol, resin, glycoside, protein,and carbohydrate. The present study thus emphasis the pharmaceutical potential of the plant and the necessity for its conservation. Morphological and Anatomical studies of *Artemisia vulgaris* L. (Asteraceae) is being studied by Sahhar *et al.*, in 2010, they explained about the morphology and anatomy of *Artemisia vulgaris* and this article helps for the how to study of morphology anatomical studies on weed plants.

Fluorescence analysis by spectrophotometer:

After several processes of chemical treatments and centrifugation the extract was subjected to scan at a wavelength ranging from 400 to 700 nm of wavelength using a spectrophotometer and those values were recorded (Table – 4). Highest absorbance for *Centella asiactica* was recorded at 500 nm, for *Phyllanthus niruri* it was 420 nm, for *Argemone mexicana* it was 500 nm and in *Amaranthus viridis* the highest absorbance is recorded at 500 nm. Highest absorbance for *Asclepias currasavicca, Atylosia scarboidesis, Cyperus iria, Portulaca oleraceae* was recorded at 500 nm. In the fluorescence analysis clearly shows that the majority of the medicinal weeds highest absorbance recorded at 500 nm and very least absorbance recorded in 700 nm for all three species except *Atylosia scarboidesis* the absorbance is at 660 nm.In the fluorescence analysis clearly shows that the majority of the medicinal weeds highest absorbance recorded at 500 nm and very least absorbance recorded in 700 nm for all the species.

A similar type of morphological studies on *O. gratissimum* was done by Chirstian, 2012 and Prabhu *et al.,* 2009. Morphological variability in holy basil was earlier reported by Malav *et al.,* 2015. Rawat *et al.,* 2016 studied morphology and anatomical features of four *Ocimum* spp. and this study is useful to correct identification, judging the authenticity of the plant and to differentiate these species from each other's while undertaking pharmacognostical characterization and evaluation. Medicinal

plants are a rich source of numerous pharmacologically active molecules. In view of this phytochemical analysis of Methanolic extract was done by Rajesh *et al.,* 2013 on *Curcuma longa.* Preliminary phytochemical evaluation of *Ocimum basilicum* and *O. tenuiflorum* was done by Venugopal *et al.,* in 2015 and find out that *Ocimum basilicum,* a novel and potential medicinal herb with sharp pungent aromatic principles. Gupta *et al.,* 2012 and Babu *et al.,* 2009 worked on morphological and anatomical features of a few plant species.

Single staining method:

The TS of *Centella asiatica* petiole shows single layered epidermis covered by cuticle. Inner cell walls of the epidermis adjoining cortex is much thickened. 2-3 layers of collenchyma tissue follow by the epidermis and there is a broad zone of parenchyma, seven vascular bundles is located in the projecting arms of the petiole. Chlorophyll pigment is seen in the outer peripheral layers of the parenchyma. Crystals of calcium oxalate are also notices in the parenchymatous zone (Fig. 4, A and B). The T. S. of *Centella asiatica* stolon (Fig. 4, C and D) shows irregularly circular in outline with tissue organization as outer epidermis, middle cortex, and inner stele. The epidermis was uni-layered and covered by a conspicuous cuticle. Underneath the epidermis, the collenchyma consisted of three cell layers. The cortex forms comparatively broad zone consisted of 8-10 layered parenchymatous tissues. Outer cortex consisted of 2-3 layers of small-sized cells followed by relatively large sized parenchymatous cells. Parenchyma cells were polygonal in shape with slightly thickened walls; cortical cells were loosely arranged to leave small air spaces (aerenchyma) in the cortical parenchyma. The vascular bundle was conjoint, collateral and open. The vascular cylinder was more than five, arranged in the form of a ring. Phloem was aligned towards the adaxial side and xylem towards the abaxial side. Stolon contained centrally located pith. Pith cells were a circular or polygonal shape.

The TS of *Phyllanthus niruri* shows thick cuticle it covers single layered barrel-shaped parenchyma epidermal cells. Next, to the epidermis, the hypodermis forms three-layered collenchyma cells. The cortex of *Phyllanthus niruri* shows secondary development. Phloem extended towards the periphery, xylem towards pith with well-developed interfascicular cambium. Metaxylem is endarch, protoxylem is exarch with large central parenchymatous pith (Fig. 5, A and B).The T.S. of *Argemone mexicana* (Fig. 5, C and D) shows a single layered epidermis they are compactly arranged without having intercellular space, stomata are present. The cortex region divided into hypodermis, parenchyma cells region and endodermis region. Hypodermis region composed from collenchyma cells with thickened corners forming a band just beneath the epidermis. In parenchyma region composed from parenchyma cells with intercellular space. Next to the parenchyma region hypodermis present in a single layer. After the hypodermis the pericycle present. In stellar region the vascular bundles arranged in the form of a distinct ring and the intrastellar ground tissues. The vascular bundles placed towards epidermis. Vascular bundles are collateral, endarch, and open type. Parenchymatous cells present in between every two vascular bundles look like rays radiating from pith they are called medullary rays and pith is the large central portion of the stem composed by parenchyma cells. The TS of *Amaranthus viridis* (Fig. 5, E, F and G) shows three to four outer epidermal parenchymatous cells with thick cuticle region with an irregular margin. Next, to the epidermis the hypodermis composed from parenchymatous cells with oil ducts. The vascular bundles show secondary growth with secondary phloem and xylem by the formation of interfascicular cambium. Many vascular bundles are present in the pith region which shows anomalous growth.

The TS of *Asclepias curassavicca* consist of single layered barrel shaped epidermis followed by 3 to 4 layered collenchymas cells in between the collenchymas cells lignin is seen in the cortex region in between cortex region and the vascular bundles vacuoles are seen inside the vacuoles few lignin are present. Vascular bundles show conjoint collateral vascular bundles the pith is present in the centre (Fig. 6A). The TS of *Atylosia scarboidesis* contains trichomes on the epidermal cells epidermal cells consist of barrel shaped cells 3 layered cortex regions consist of collenchyma's cells secondary vascular bundles seen in metaxylem followed by protoxylem. The centered pith contains parenchymatous cells (Fig. 6B).

The TS of *Cyperus iria* stem consist of single layered epidermal cells below the epidermal region cortex region is present where vascular bundles are scattered two metaxylem followed by one protoxylem which is surrounded by phloem. Inside the pith region palisade parenchymatous cells

surrounded to the vacuoles is observed (Fig. 6, C and D). The TS of stem of *Portulaca oleraceae* consist of barrel shaped epidermal cells below the epidermal region cortex is formed by collenchymas cells with 7 to 8 layered cells inside the collenchymas cells calcium oxalate crystals are found. 12 to 13 Vascular bundles are endarch in condition and surrounds the parenchymatous pith region in the centre (Fig. 6E)

Double staining method (Safranin-Fast Green):

In the section of *Amaranthus viridis* (Fig. 7, A-C) and *Argemone mexicana* (Fig. 7, D-F) shows well developed double staining differentiate and in that section we differentiated cells like phloem and xylem tissues because of two different colors. In this section shows different colors because the components of cell walls of xylem, phloem, and other cells are different. But in the *Phyllanthus niruri and Centella asiatica* stem does not show well colour differentiated the double staining method is not well applicable i.e. there is no well differentiated in cell colors (Fig. 7, A-C). The double staining technique is not successful for a few plant parts. The information of present work is important, as it helps in the identification of these species and contributes to its quality control, and evaluation.

In the section of *Asclepias currasavicca* (Fig. 8A) *Cyperus iria* (Fig. 8B), and *Atylosia scarboidesis* (Fig. 8C) shows well differentiated double stained cells such as phloem and xylem (vascular bundles) in purple colour and pith and cortex region in pinkish red colour. In the *Atylosia scarboidesis* cortex and secondary vascular bundles are in pinkish red colour in *Cyperus iria* phloem cells is in red in colour and cortex region is in purplish colour.

But in *Portulaca oleraceae* double staining is not well differentiated. In this section does not shows different colors clearly because the components of cell walls of xylem, phloem, and other cells are not taken stain properly hence the double staining method is not well applicable i.e. there is no well differentiated in cell colors (Fig. 8D). The double staining technique is not successful for a few plant parts. The information of present work is important, as it helps in the identification of these species and contributes to its quality control, and evaluation.

Arora and Saini in 2018 examine the anatomical characters of the whole plant of *C. decumbens* by double staining method for identification. The TS of root, stem, leaf, and flower have been examined and analyzed. Manik in 2013 studied the transverse section of stem, root, and leaf of *Amaranthus spinosus* with the help of double staining technique. Lee and Black (1955) studied anatomical studies of *Trifoliumin carnatum* infected by the wound-tumor virus by using different staining procedure. There is no record for using double staining technique to these four medicinal weeds. Anatomical and histological study of stem, root, and leaf of the medicinal plant of *Amaranthus spinosus* was being studied by Manikin (2013) using double staining. The anatomy of stem and roots showed cellular differentiation. Both the stem and root showed secondary growth. In the stem, the vascular bundle pattern is conjoint, collateral and endarch type; whereas root showed conjoint, collateral and exarch type of vascular bundle. The powdered drug, treated with different chemicals and its extracts with different solvent showed colour changes when illuminated with UV light. A comparative study of morphological and anatomical structures of four *Ocimum* species in Uttarakhand, India studied by Rawat *et al.*, in 2016. In this article, the traditional systems of medicine along with a holistic approach, different parts of basil (*Ocimum* spp.) have been prescribed for the treatment of various ailments. Morphological and anatomical characters play a vital role in plant-based crude drug identification and standardization. This study is useful to correct identification judging the authenticity of the plant and to differentiate these species from each other's while undertaking pharmacognostical characterization and evaluation.

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