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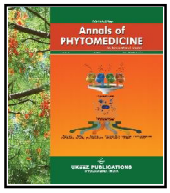

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Pharmacognostic evaluation of some Indian medicinal plants from Maharashtra

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Abstract

Plants are colossal source for different phytomolecules which in turn exhibit varied pharmacological properties. Objective of this study is identification of such potential plants. It becomes mandatory to study all the pharmacognostical and physicochemical parameters of these plants before they are used in any pharmaceutical formulations. It also becomes helpful in differentiating these plants from other similar species and adulterants.

Keeping this in mind, the pharmacognostic characteristics of the leaves of some Indian medicinal plants; namely, *Aegle marmelos* (L.) Correa, *Cynodon dactylon* (L.) Pers., *Annona squamosa* Linn. and *Moringa oleifera* Lam. were examined. Different characteristics like, palisade ratio, vein islet numbers, stomatal index (upper and lower surfaces of the leaf), *etc*., were studied. The leaves of the plants under study, *viz*., *A. marmelos*, *C. dactylon*, *A. squamosa* and *M. oleifera* showed typical pharmacognostic characteristics. These outcomes will help in setting the pharmacognostic standards for identifying the plant. It will also be useful for purity, classification and overall quality of the plant, which is gaining importance in research, based on plant drugs.

1. Introduction

In the traditional medicine, the plants in crude form are utilized for their therapeutic effects against different human ailments. However, the crude drugs often get adulterated due to some morphological similarities with the other similar species and/or lack of correct identification methodology, which apparently results in the loss of drug efficacy. Thus, for gaining maximum benefits and safe use of plant based natural health products, it becomes necessary to ensure correct identification of the herbal drug. Without proper identification and characterization, the safe use of quality products cannot be guaranteed (Ahmed *et al.*, 2009).

Herbal medicines play an important role in the healthcare systems of many people across the world and are extensively studied for their effects on several diseases (Singh *et al*., 2022; Sabeena *et al*., 2022; Afroz *et al*., 2022). The adulteration of the samples sold in the market has become one of the greatest drawbacks in promotion of herbal products (Dubey *et al.*, 2004). Dehydrated products available in the market are usually difficult to categorize, as many of the useful diagnostic characters are lost during drying and processing. Correct identification of the traded herbal drugs by taxonomists is also an important task. Several local names for the same plant species in different areas may contribute to the misperception regarding the selection and appropriate usage of the original genuine drug. Another problem is due to superficial similarity of plant species within the same tribe or family (Khan *et al.*, 1996). Usage of different plants from different species for one ailment may also lead to the problem of adulteration (Shinwari *et al.*, 2002).

Thus, quality control of herbal drugs and its constituents plays a major role for the safe acceptability of the medicinal plants in the modern system of medicine. However, sometimes such stringent quality control profiles for herbal raw and finished drugs are not followed in the industry, which leads to the compromise in the quality. With the use of new analytical tools and sophisticated instrumentation, feasible quality assurance profile for a crude drug or its constituent can be achieved (Santosh *et al*., 2022; Kokate *et al.*, 2009).

Over the years, there has been a systemic changes in the evaluation. Previously, the only method to identify the crude drugs was by comparing with the standardized reports available. But, now due to availability of chemical knowledge of the crude drugs, the evaluation also includes different methods for estimating the active bioconstituents in the crude drugs. Due to the advanced instrumentation like, efficient separation techniques, it is now possible to carry out qualitative and quantitative evaluation of a crude drug. Pharmacological evaluation is needed to understand the biological behaviour of the crude drug extracts.

Morphological characteristics and histological, chemical and biological studies aids in the proper identification of crude drugs. Each and every plant has its unique external and internal body morphology, which can become markers for their identity.

The present work has been undertaken to establish the various pharmacognostical parameters of the leaves of *A. marmelos*, *C.*

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dactylon, *A. squamosa* and *M. oleifera* in fresh and powdered form using standard procedures from Indian Herbal Pharmacopoeia, United States Pharmacopoeia, WHO, The British Pharmacopoeia Vol. IV, Indian Pharmacopoeia, Vol. I and II (The British Pharmacopoeia, 2009; Mukherjee, 2002; Indian Herbal Pharmacopoeia, 1998; WHO, 1998; United States Pharmacopoeia, 1994), which could serve as the measure of authentication and quality control for commercial samples.

2. Materials and Methods

Leaves of *Aegle marmelos* (L.) Correa, *Cynodon dactylon* (L.) Pers., *Annona squamosa* Linn.*and Moringa oleifera* Lam.were collected from Mumbai and Talegaon Dabhade (district - Maval, Pune). Authentication of the plant samples *Aegle marmelos* (L.) Correa (Acc. No.-08649, 08652), *Cynodon dactylon* (L.) Pers. (Acc. No.-83143), *Annona squamosa* Linn. (Acc. No.- 102268, 00469) and *Moringa oleifera*Lam. (Acc. No.-12778, 12781) was done by Dr. Rajendra Shinde, Blatter Herbarium, St. Xavier's College, Mumbai.

2.1 Pharmacognostic study

The external morphological characteristics of the leaves of *A. marmelos, C. dactylon, A. squamosa and M. oleifera*were studied in fresh form (Figure 1). For the microscopic study of the plant powders, each plant material was dried and processed.

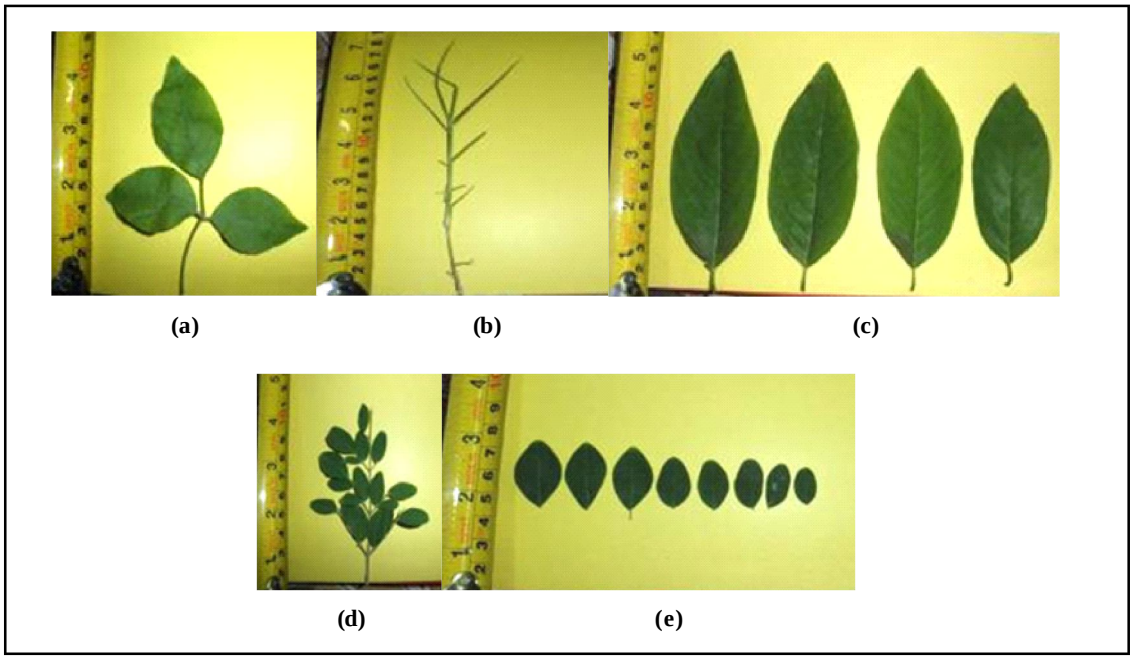

Figure 1: External morphology of the leaves of (A) *Aegle marmelos* (L.) Correa., (B) *Cynodon dactylon* (L.) Pers., (C) *Annona squamosa* Linn. and (D) and (E) *Moringa oleifera* Lam.

2.2 Macroscopic studies of fresh plant leaves

External morphology of the said plant leaves were studied by naked eye and with the help of dissecting microscope.

2.3 Microscopic analysis of fresh plant leaves

The fresh leaves of *A. marmelos, C. dactylon, A. squamosa and M. oleifera*were separated and washed thoroughly. Thin transverse sections of the leaves were taken and temporarily double stained with saffranin and hematoxylene by the standard procedure. The slides were observed under microscope with different magnifications, anatomical characteristics of the four leaves were noted down and photomicrographic records were made.

2.4 Quantitative microscopy of fresh plant leaves

2.4.1 Determination of stomatal index (Dinesh *et al.*, 2012)

It is the percentage proportion of the epidermal cells of a leaf, which have been converted into stomata. It is defined as the ratio of the count of stomata from a unit area to the count of epidermal cells in that area. The count of stomata is age dependant, *i.e.*, it varies as the age of the same plant changes but the stomatal index remains the same for a given species of the plant. It helps to distinguish between two species of the same genus. It can be determined even in powder form of the leaf. It helps to determine purity of the drug sample under study and hence, is used in pharmacognosy tests to find out adulterations in the samples.

About 5 x 5 mm size leaf fragments of the plants under study were placed in test tube. 5 ml of chloral hydrate solution was added and then boiled for 15 min at 100°C or till the fragments became clear and transparent. A fragment was transferred to a microscopic slide mounted, in chloral hydrate solution and a small drop of glycerol, ethanol solution on one side of the cover glass to prevent the preparation from drying. Examination was done at 240X. A microscopial drawing apparatus was attached to the eyepiece. A cross mark (x) was marked on the paper for each epidermal cell and each stomata was represented by a circle (o).

The stomatal index was obtained by using the following formula:

$$\text{Stomatal index (I)} = \frac{S}{S + E} \times 100$$

Note: S = Total count of stomata per unit area

E = Total count of ordinary cells in the same unit area

For each leaf sample, minimum of ten determinations were done and the average of the index was calculated.

2.4.2 Palisade ratio determination (Dinesh *et al.*, 2012)

The average of the number of palisade cells under each epidermal cell is termed as the palisade ratio. Palisade ratio can be calculated by using following formulae:

$$\text{Palisade ratio} = \frac{\text{Total number of palisade cells}}{\text{Total number of epidermal cells}}$$

Palisade ratio is fairly constant for a given species. It helps to distinguish between two species of the same genus. It is a parameter for pharmacognosy tests to find adulteration in the samples and helps to determine purity of the drug understudy.

A leaf fragments of the plants under study (around 5 × 5 mm in size) were placed in a test tube. 5 ml of chloral hydrate solution was added and then boiled for 15 min at 100°C or till the fragments comes outclear and transparent. A fragment was transferred to a microscopic slide mounted; the upper epidermis in chloral hydrate solution and a small drop of glycerol solution was put on one side of the cover glass to avoid the preparation from getting dried. Examination was done at 240X. A microscopy drawing apparatus was attached to the eyepiece was marked on the drawing paper. Four nearby epidermal cells on paper were traced; gently focused downward to bring the palisade into view and sufficient palisade cells were traced to cover the area of the outlines of the four epidermal cells. When the cell is intercepted, it was counted only when more than half of it was within the area of epidermal cells. The average number of palisade cells beneath one epidermal cell dividing the count by 4 was calculated.

Note:The veins of monocot leaves are parallel to one another throughout the length of the leaf and they do not branch. There may or may not be a mid-vein present, but all other veins are of a single order. Stomata can be seen on both the leaf surfaces; however, the palisade layer is seen to be indistinct from the spongy mesophyll layer in monocots. Cells in both layers are roughly isodiametric. Hence, palisade ratio, is not applicable to monocot leaves due to a lack of consistent differentiation within the mesophyll the analysis of leaf constants (quantitative microscopy) was, therefore was not performed for *Cynodon dactylon* (L.) Pers. (Indian Pharmacopoeia, 1996).

2.4.3 Vein islet number determination (Srinivasa *et al.*, 2008)

It is defined as the number of vein islets per square millimeter of the leaf surface. The area of the leaf sample under consideration is preferably taken from the lamina midway between the midrib and margin. The count of vein islet per square millimeter is termed as vein islet number. This count per unit area of the leaf remains same. vein islet number is the leaf constant, which can be used fairly as a diagnostic character in the evaluation of leaf drugs. This number is fairly constant for a given species, irrespective of size of the leaf and does not change with the age of the plant. It helps to distinguish between two species of the same genus (Golatkar *et al.*, 2011).

Pieces of leaf lamina were taken with an area of four square millimeters from the central portion of the lamina and not including the midrib and the margin of the leaf. A test-tube with chloral hydrate was taken and the pieces of lamina were added to it. Test-tube was boiled for 30 to 60 min or till the sample is clear and then mount with glycerol solution was prepared or, if chosen, it can be stained with saffranin solution and the mount be prepared with Canada balsam. The stage micrometer on the microscope was placed. Examination was done at 240X by drawing a line representing 2 mm on sheet of paper by means of a microscopy drawing apparatus and then a square was constructed on the line which representedan area of four square millimeters. The paper was moved in such a way that the square was observed in the centre of the field of the eyepiece. The slide with cleared leaf sample was positioned on the microscope stage and haggard in the veins and veinlets included within the square, completing the outlines of those vein-islets, which overlaped the two sides of the square. The count of vein-islets within the square including those overlapping on two sides and eliminating those intercepted by the other two sides were counted. The result obtained is the vein islets in four square millimeters. For each sample of leaf, not fewer than three determinations and the average count of vein islets per square millimeter was calculated.

2.4.4 Fluorescence analysis and powder microscopy of the dry plant powders (Kokshi *et al.*,1958)

For fluorescence analysis, the plant leaf powders were examined directly under UV light and day light by adding different reagents.

The powder microscopy was done by staining the dry plant powders with dilute aqueous saffranin for two minutes. At the end of two minutes, the stained material was washed to remove the excess stain and mounted in dextrin plasticizer xylol (DPX) to make a permanent mount. The slides were studied under light microscope at different magnifications and photomicrographs were taken.

3. Results

3.1 Pharmacognostic study

3.1.1 Macroscopic analysis of fresh plant leaves

The observations and the results of the pharmacognostic study of the leaves of *A. marmelos*, *C. dactylon*, *A. squamosa* and *M. oleifera* are discussed as follows:

1. ***Aegle marmelos* (L.) Correa.:** The leaves of *A. marmelos* are alternate, pale or dark green, compound, trifoliate; glabrous or grey-pubescent, smooth, alternate, membranous, odd pinnate of 3 leaflets (sometimes 5 leaflets present); lateral leaflets opposite and nearly sessile, ovate-lanceolate, 4.5 cm long and 2.2 cm wide; terminal leaflet with long rachis, 4-6 cm long and 2-5 cm broad, ovate-lanceolate, entire or crenate, acute, reticulate, covered with glands, rachis 1.5 to 3 cm long, petiole not winged about 3.2 cm long; odour like lemon, taste sweetish, aromatic, slightly like lemon. New foliage is glossy and pinkish-maroon. Mature leaves gave an unpleasant odour when bruised.
2. ***Cynodon dactylon* (L.) Pers.:** The leaves of *C. dactylon* are simple, alternate and ribbon shaped, long, 2 to 10 cm in length and 1.25 to 3 mm in width, narrowly linear or lanceolate, finely acute more or less glaucous, smooth, soft usually clearly

distichous in the barren shoots and at the base of the stems; sheath light, glabrous or occasionally bearded, entire lower leaves usually flat, upper complicate, parallel veined, with sheathing leaf bases attached at the node and surrounding the internode like a tube to varying lengths; often splitted at base; ligule, a projecting portion of sheat, very short, fine ciliate rim; hairy and erect.

3. ***Annona squamosa* Linn.:** The leaves of *A. squamosa* Linn. occur singly, are upto 6-17 cm in length and 3-6 cm in breadth; leaf opposite; lanceolate or oblong lanceolate; acute or subacute; pellucido-punctate, pale green on both surfaces and glabrate or nearly so; glabrous above; glaucous and pubescent beneath when young; sides sometimes marginallyuneven, no teeth on edges, subtly hairy when young, dotted minutely when observed with a lens with thin, dark green on upper surface and on underside it looks pale blue-green, short or long pointed apex is seen ; base short pointed or rounded; venation pinnately reticulate; lateral nerves 8 to 11 pairs; petioles 6-12 mm in length, green, sparsely pubescent.

4. ***Moringa oleifera* Lam.:** Leaves were like feathers, pale green, compound, tripinnate, 30-60 cm in length, with many small leaflets, 1.2-2 cm in length, 0.5-1.0 cm in width, lateral ones somewhat elliptic, terminal one ovate or obovate and marginally larger than the lateral ones, rounded or narrowed at base and obtuse at apex; odour and taste not distinct; Leaves were seen to be alternate with opposite pinnae, spaced outaround 5 cm away up the central stalk, typically with a second lot of pinnae, also opposite, bearing leaflets in opposite pairs. Leaves are variable in size and shape and show smooth dark green colour on the upper side and pale green colour underneath.

3.1.2 Microscopic analysis of fresh plant leaves

1. ***Aegle marmelos* (L.) Correa.:** Transverse section (T.S.) of the leaf sample shows a single layered upper epidermis made of cells which are polygonal and barrel shaped with straight thick anticlinal walls covered with thick cuticle, cells of lower epidermis are smaller in size, stomata anomocytic, present on both the surfaces but abundant on lower surface; mesophyll differentiated into 2 or 3 layers of small palisade cells which are continuous on midrib and very compactly arranged spongy parenchyma containing chloroplast; large, circular secreatory cannals surrounded by single layer of epithelial cells present in mesophyll.

   Midrib slightly pronounced towards lower surface; shows single layered epidermis covered with thick cuticle, cells of upper epidermis are bigger in comparison to lower surface; lower epidermis of midrib differentiated by 2 or 3 layers of collenchyma, remaining ground tissue is parenchymatous; meristele are arc shaped, vascular bundle consists of radially arranged xylem and encircled by phloem, pericycle represented by patches of sclerenchymatous fibres. Rare trichomes, found mainly on the lower surface of mid-rib region, unicellular and 399 - 696 µm long (Figures 2A and 2B).

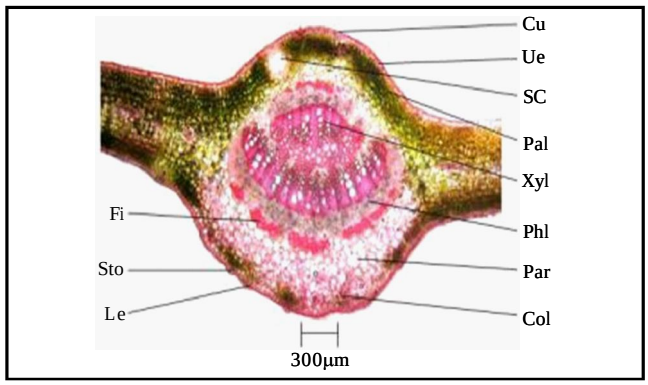

Figure 2 (A): Transverse section of the midrib portion of the leaf of *Aegle marmelos* (L.) Correa. (Fi: Fibers; Sto: Stomata; Le: Lower epidermis; Col: Collenchyma; Par: Parenchyma; Phl: Phloem; Xyl: Xylem; Pal: Palisade cells; SC: Secretary Canal; Ue: Upper epidermis; Cu: Cuticle).

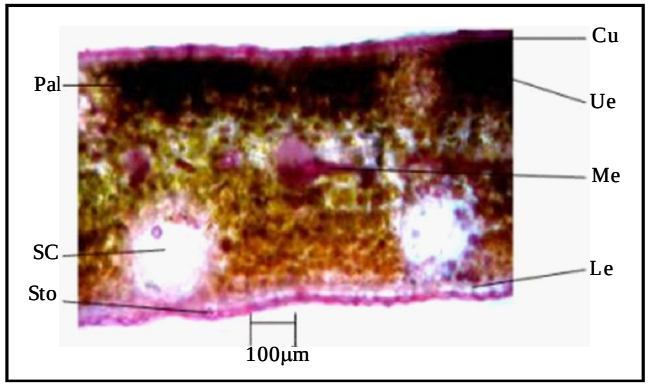

Figure 2(B): Transverse section of the lamina portion of the leaf of *Aegle marmelos* (L.) Correa. (Sto: Stomata; Le: Lower epidermis; Pal: Palisade cells; SC: Secretary Canal; Ue: Upper epidermis; Cu: Cuticle; Mes: Mesophyll).

2. ***Cynodon dactylon* (L.) Pers.:** Transverse section (T.S.) of the leaf is ribbon shaped with a centrally located conspicuous meristele each lying in the primary vein alternating with rows of 3 to 4 smaller sized meristele placed almost at equal distances in the secondary veins (Figures 3A and 3B).

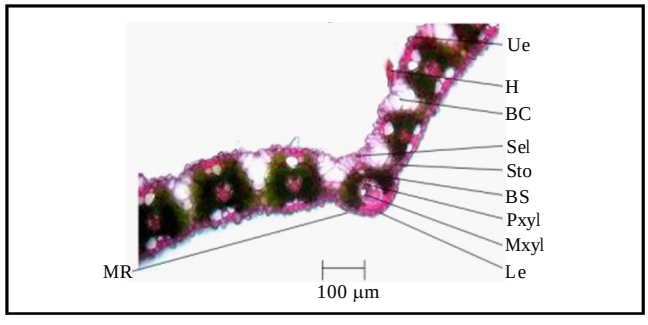

Figure 3(A): Transverse section of the midrib portion of the leaf of *Cynodon dactylon* (L.) Pers. (H: Hair; Sto: Stomata; Ue: Upper epidermis; BC: Bulliform Cells; Scl: Sclerenchyma; Pxyl: Protoxylem; Mxyl: Metaxylem; BS: Bundle Sheath; Le: Lower epidermis; MR: Midrib Region).

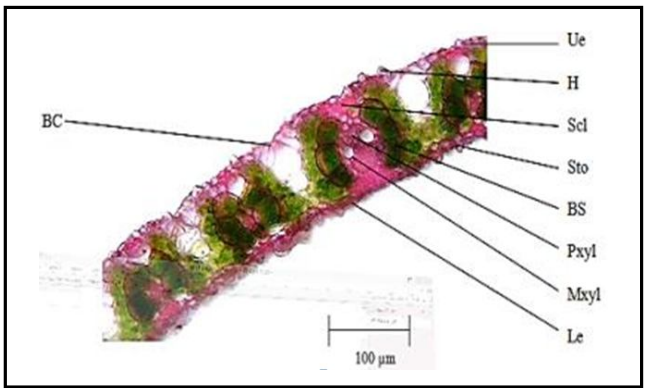

Figure 3(B): Transverse section of the lamina portion of the leaf of *Cynodon dactylon* (L.) Pers. (H: Hair; Sto: Stomata; Ue: Upper epidermis; BC: Bulliform Cells; Scl: Sclerenchyma; Pxyl: Protoxylem; Mxyl: Metaxylem; BS: Bundle Sheath; Le: Lower epidermis).

Transverse section (T.S.) of the leaf also shows presence of lamina with nearly square to oval epidermis having irregularly cutinised outer wall. Dorsal side showed presence of bulliform cells grouped together, which lie under a clear groove in between the veins. These are thin walled and they lack chlorophyll and spread deep into the mesophyll.Mesophyll is not differentiated into palisade and spongy parenchyma; row of vascular bundles closelysimilar, except that the median bundle is greater; bundle sheath is single, and it has thin-walled more or less isodiametric parenchyma cells containing chloroplast; mesophyll tissue is broken by 1 or 2 thin-walled colourless cells, which spreads from bundle sheath to the thin walled parenchymatous band of stereome near upper and lower epidermis.

3. ***Annona squamosa* Linn.:** Transverse section of the leaf shows a dorsiventral nature. The section is broadly divided into the lamina and the mid-rib. The lamina of the leaf shows clearly three regions, *viz*., upper epidermis, lower epidermis and the mesophyll. The upper layer,the epidermis is made of single layered of straight walled cells which are more or less rectangular in shape. It is seen to be continuous with the coat of wavy cuticular thickening and simple or stellate hairs. Upper epidermis shows absence of stomata. Whereas the lower epidermis seems continuous with a wavy cell wall which isfrequently interrupted by the occurrence of stomata lower epidermis also shows presence of simple or stellate hairs. However, the cuticular thickening is not very well represented. Single stellate, prismatic, square or cluster crystals are present on both the upper and lower surfaces of some cells.The mesophyll tissue is made up of palisade and spongy parenchyma. The upper palisade layer is made up of two layers of vertically arranged compact tubular palisade cells containing chloroplastids. Some starch grains are seen in the palisade cells. The lower bulk of the mesophyll tissue is made up of spongy tissue, which is made up of oval and circular spongy parenchyma cells, enclosing intercellular spaces filled with air. In the spongy region, there are scattered groups of sclereids with thick cell walls, which provide mechanical stability to the leaf. The spongy parenchyma cells also contain chloroplasts and starch grains. Vascular strands show closed collateral bundles which get distributed in a reticulate fashion in the lamina. These specialized structures carry water and food to the leaf. In the midrib, which is clearly, below the upper epidermis and above the lower epidermis there are one or two layers of collenchyma cells, which constitute the hypodermis. Beneath the upper collenchyma and above the lower collenchyma, medulla cells are present. These are parenchymatous enclosing lysigenous secretory cavities that help in the storage of mucilaginous content. In the upper part of the medulla, groups of sclereids are present, which also provide mechanical strength. Towards the upper epidermis, the vascular bundles are distributed in the form of an arc. The number of vascular cylinders varies from around 8 to 12. Closed and collateral vascular bundles are seen, exarch with xylem pointing downwardsand vessels with reticulate thickening. The phloem is towards the lower epidermis and consists of sieve tubes, companion cells and phloem parenchyma. Below the phloem, there are sclerenchymatous fibre strands capping each vascular bundle which also provide mechanical support to the leaf. Parenchymatous tissue encloses the central part of the vascular bundle having innumerable starch grains connecting the central part of the medulla are connected to the outer part of the midrib by the vascular bundles which are radially traversed by interfascicular or intervascular parenchyma(Figures 4A and 4B).

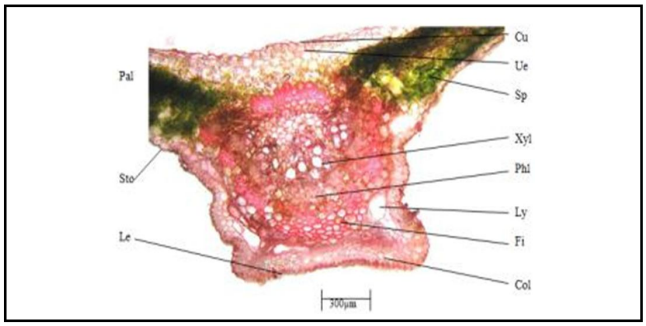

Figure 4 (A): Transverse section of the midrib portion of the leaf of *Annona squamosal* Linn. (Fi: Fibers; Sto: Stomata; Le: Lower epidermis; Col: Collen chyma; Ly: Lysogenous secretary cavities; Phl: Phloem; Xyl: Xylem; Sp: Spongy cells; Ue: Upper epidermis; Cu: Cuticle; Pal: Palisade cells).

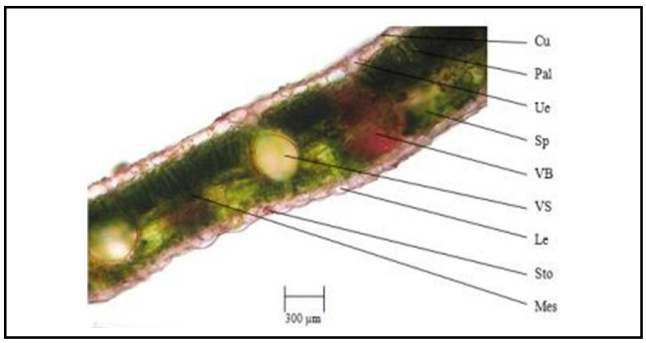

Figure 4 (B): Transverse section of the lamina portion of the leaf of *Annona squamosal* Linn. (Mes: Mesophyll; Sto: Stomata; Le: Lower epidermis; VS: Vascular space; VB: Vascular bundle; Sp: Spongy cells; Ue: Upper epidermis; Cu: Cuticle; Pal: Palisade cells).

4. ***Moringa oleifera* Lam.:** Transverse section (T.S.) of the leaf sample was taken. It shows dorsiventral structure; epidermal layer and showed presence of unicellular hair on both the surfaces; palisade is single layered; parenchyma is spongy with two to three layers; central region occupied by a crescent shaped, collateral vascular bundle surrounded by two to four layers of collenchymatous cells; mesophyll and collenchymatous cells showed the presence of rosette crystals of calcium oxalate; stomata anomocytic, present on both the surfaces but more on lower surface (Figure 5A and 5B).

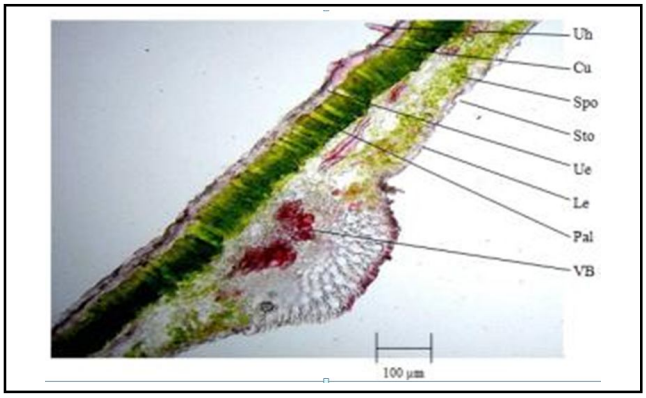

Figure 5(A): Transverse section of the midrib portion of the leaf of *Moringa oleifera* Lam. (Uh: Unicellular hair; Sto: Stomata; Le: Lower epidermis; Sp: Spongy cells; Ue: Upper epidermis; Cu: Cuticle; Pal: Palisade cells; VB: Vascular bundle).

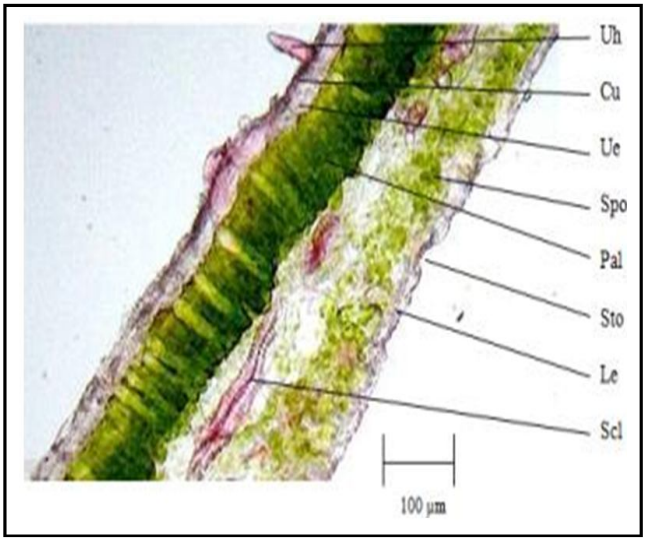

Figure 5(B): Transverse section of the lamina portion of the leaf of *Moringa oleifera*Lam. (Uh: Unicellular hair; Sto: Stomata; Le: Lower epidermis; Sp: Spongy cells; Ue: Upper epidermis; Cu: Cuticle; Pal: Palisade cells; Scl: Sclereid).

3.1.3 Quantitative microscopy of the plants under study

The results of the quantitative microscopic analysis (determination of the leaf constants) of the fresh leaves of *A. marmelos, A. squamosa and M. oleifera* are listed in Table 1.

Table1: Quantitative microscopy of the leaves of *A. marmelos, C. dactylon, A. squamosa and M. oleifera*

S. No.	Plant sample	Stomatal index		Palliade ratio (under one epidermal cell)	Vein islet number per square mm
		Upper epi.	Lower epi.		
1.	*Aegle marmelos* (L.) Correa.	4 – 8	8.5 - 11.5	1.5 – 2.5	9 - 18
2.	*Annona squamosa* Linn	-	11.5 - 14.5	6 - 8	12-17
3.	*Moringa oleifera* Lam.	6.5 - 9.5	10 – 15	5 – 10.5	5 – 8

3.1.4 Fluorescence analysis of the plant leaf powders

The fluorescence characters of the leaf powders of *A. marmelos, C. dactylon, A. squamosa and M. oleifera* are as charted in the subsequent Tables 2, 3, 4, 5.

Table 2: Showing the effect of different chemical reagents on the fluorescence behaviour of crude drug powder of *A. marmelos*

S. No.	Treatment given	Day light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Distilled water	Light yellow	Light green	Fluorescent dark green
2	Alcohol	Yellow	Yellow	Fluorescent white
3	Acetic acid	Green	Green	Yellowish green
4	1N Hydrochloric acid	Yellow ochre (Yolk colour)	Light green	Fluorescent light green
5	2N Sulphuric acid	Green	Green	Fluorescent dark green
6	50% Nitric acid	Light green	Light green	Fluorescent green
7	10% Sodium hydroxide	Yellowish green	Green	Fluorescent green

Table 3: Showing the effect of different chemical reagents on the fluorescence behaviour of crude drug powder of *C. dactylon*

S. No.	Treatment given	Day light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Distilled water	Transparent	Transparent	Fluorescent white
2	Alcohol	Yellowish green	Green	Fluorescent light orange
3	Acetic acid	Light green	Light green	Fluorescent light orange
4	1N Hydrochloric acid	Transparent	Transparent	Fluorescent green
5	2N Sulphuric acid	Light green	Fluorescent dark green	Fluorescent light green
6	50% Nitric acid	Off white	Pale green	Fluorescent light green
7	10% Sodium hydroxide	Greenish yellow	Light green	Fluorescent Green

Table 4: Showing the effect of different chemical reagents on the fluorescence behaviour of crude drug powder of *A. squamosa*

S. No.	Treatment given	Day light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Distilled water	Off white	Light green	Fluorescent light green
2	Alcohol	Dark green	Dark green	Fluorescent dark orange
3	Acetic acid	Olive green	Green	Fluorescent dark orange
4	1N Hydrochloric acid	Light yellow	Light green	Fluorescent green
5	2N Sulphuric acid	Green	Green	Fluorescent green
6	50% Nitric acid	Light yellow	Light green	Fluorescent green
7	10% Sodium hydroxide	Brown	Green	Fluorescent dark green

Table 5: Showing the effect of different chemical reagents on the fluorescence behaviour of crude drug powder of *M. oleifera*

S. No.	Treatment given	Day light	Ultra violet light (254 nm)	Ultra violet light (365 nm)
1	Distilled water	Yellowish green	Light green	Fluorescent green
2	Alcohol	Light green	Green	Reddish orange
3	Acetic acid	Olive green	Olive green	Fluorescent dark orange
4	1N Hydrochloric acid	Light yellow	Light green	Fluorescent green
5	2N Sulphuric acid	Green	Light green	Fluorescent yellowish green
6	50% Nitric acid	Light yellow	Green	Light green
7	10% Sodium hydroxide	Yellowish green	Light green	Fluorescent light green

3.1.4 Powder microscopy of the plant leaf powders

1. ***Aegle marmelos* (L.) Correa.:** The leaf powder is bright green in colour and shows following diagnostic features, fragments of epidermal cells with anomocytic stomata; stone cells; unicellular trichomes; fragments of sclerenchymatous fiber patches and spiral thickening pitted vessels (Figure 6).

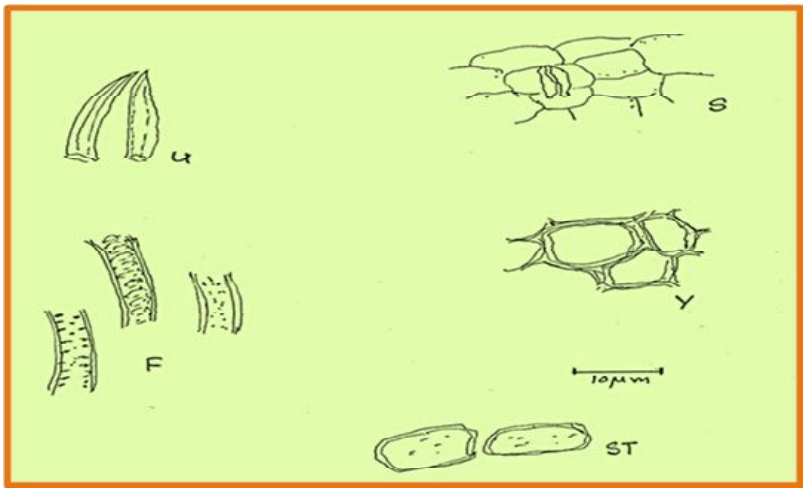

Figure 6: Powder microscopy of the leaf powder of *Aegle marmelos* (L.) Correa. (S: Epidermal cells with stomata; F: Sclerenchymatous fibers; ST: Stone cells; U: Unicellular trichomes; Y: Yellow coloured polygonal sclerenchymatous cells.

2. ***Cynodon dactylon* (L.) Pers.:** The leaf powder is yellowish-green in colour. It shows simple pitted, scalariform, spiral and annular vessels; short lignified, thick walled, sharppointed fibres, paracytic stomata; in surface viewstretched epidermis is seen with long rectangular / square cells having sinuous walls; simple and compound type of starch grains, admeasuring 1-3 ¼ m in diameter. Oil globules seen in upper and lower epidermis (Figure 7).

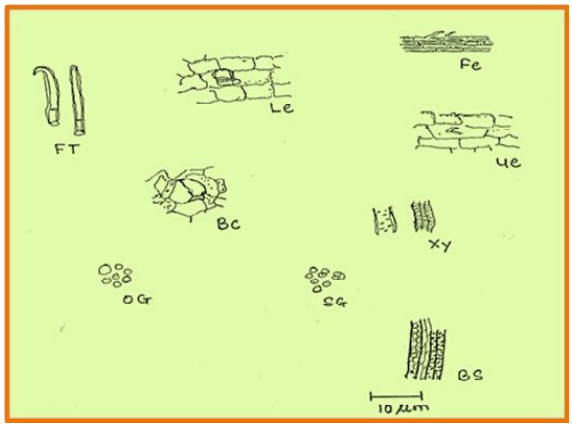

Figure 7: Powder microscopy of the leaf powder of *Cynodon dactylon* (L.) Pers.

(FT: Fragments of long trichomes; Le: Lower epidermis; OG:Oil globules; BC: Bulliform cells; Fe: Fragments of epidermis with prickle hair; SG: Single and compound starch grains; Xy: Xylem vessels; BS: Bundle sheath cells along with vascular strands; Ue: Upper epidermis).

3. ***Annona squamosa* Linn.:** The leaf powder sample is dark greyish green or dull bottle green in colour with unpleasant odour. Taste was mucilaginous. Microscopy of the leaf powder sample showed many sickle shaped unicellular and also multicellular and lignified trichomes which were stellate, sometimes broken and rarely collapsed. Paracytic or rubiaceous type of stomata with irregularly shaped epidermal cells were observed. Prismatic and cluster calcium oxalate crystals were present in the sheath of cells around the fibres. A number of stone cells, which were lignified were seen isolated or in groups. Concentric starch grains were also seen. Apart from these characters, oil sacs were seen at intervals. There were also a few lignified vessels (Figure 8).

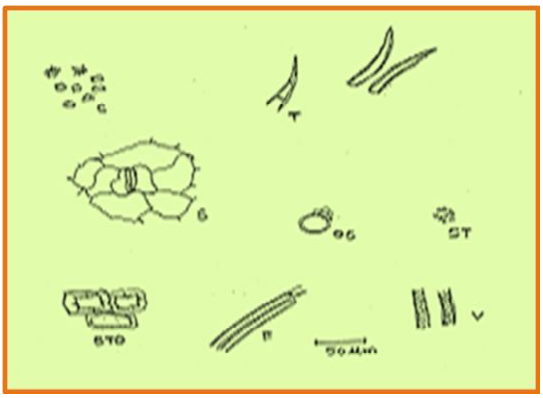

Figure 8: Transverse powder microscopy of the leaf powder of *Annona squamosa* Lam.

Linn (C: Crystals; STO: Stone cells; T: Trichomes; O/S: oil sac and stomata; ST: Starch; V: Vessels; F: Fibres).

4. ***Moringa oleifera* Lam.:** The leaf powder is bright, green in colour; showed fibres with blunt tip; prismatic and rosette shaped crystals of calcium oxalate, few starch grains and sclereids (Figure 9).

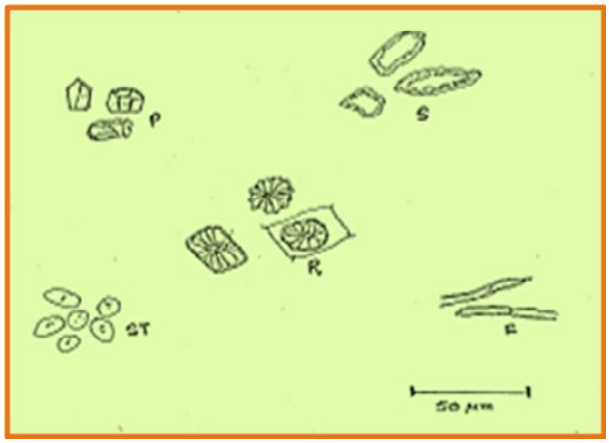

Figure 9: Powder microscopy of the leaf powder of *Moringa oleifera* Lam.

(P: Prismatic crystals of calcium oxalate; ST: Starch grains; R: Rosette crystals of calcium oxalate; S: Sclereids; F: Fibres).

4. Discussion

Plants are colossal source for various phytomolecules showing various pharmacological properties. Plants have crucial status in contemporary therapeutical systems because of multiple reasons, *viz.*, safety and less side effects (Sharma *et al.*, 2021). Hence, it is necessary to study the pharmacognostic characteristics of the plant before it can be used for research or for preparing pharmaceutical formulations. Furthermore, it also helps in differentiating the target plant from other related species and various adulterants.

Keeping this in mind, the Pharmacognostical characteristics of the leaf of the plants, *A. marmelos, C. dactylon, A. squamosa* and *M. oleifera* were examined, in the present study. The study of macromorphological and the microscopic features of these plants may serve an important role in assigning botanical standards when investigated by pharmacognosists.

Microscopic evaluation for identification of medicinal herbs is a crucial tool and is one of the necessary factor in modern monograph. In this regard, the important microscopic features of the leaves of the plants - *A. marmelos, C. dactylon, A. squamosa* and *M. oleifera* have been documented in this section such as T.S. of the fresh leaves of the said plants, the study of the powder characters of the leaf powders and the quantitative microscopic studies of leaves. The plants under study, *viz.*, *A. marmelos, C. dactylon, A. squamosa* and *M. oleifera* are used extensively in the traditional system of medicine for the treatment of number of ailments (Singh *et al.*, 2020; Khatun and Das, 2020; Swarankar *et al.*, 2019; Ugwoke *et al.*, 2017; Shendye and Gurav, 2014; Saha, 2011). Although, these are very common plants having less possibilities of adulteration but to get highest efficacy of an herb drug or its finished product cent per cent genuine plant material should be the source material.

All these above said characters as well as the fluorescence analysis of the leaf powders reflect the diagnostic features of the said plants/ plant parts in fresh and/or powdered forms and can be used to check adulteration. Studies on quantitative microscopy can provide us with important source of facts and can offer suitable standards for the determination of the overall quality of these plant materials for future research.

The fluorescence colour is highly specific for each compound. Fluorescence can be seen in a non-fluorescent compound, if it is mixed with impurities that are fluorescent. The fluorescent method is sufficiently sensitive and enables the exact and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al.*, 2006; Kala *et al.*, 2010). Similar studies were done by Tripathi *et al*. (2019) using bark powder of *A. marmelos*.

In the present study, fluorescence analysis of the leaf powder of *A. marmelos, C. dactylon, A. squamosa* and *M. oleifera* showed the presence of fluorescence compounds which would help as valuable information for the scientists working in the field of research on the medicinal properties of these plants.

The findings of the pharmacognostic study of the leaf/leaf powders of *A. marmelos, A. squamosa* analysed in present study were complying with the reports of Vijaylakshmi, *et al*. (2016); Siddique *et al*. (2010) and Tiwari *et al*. (2010) for the leaves of *Aegle marmelos* (L.) Correa. and Bavage *et al*. (2020); Dinesh Kumar *et al*. (2005) for the leaves of *Annona squamosa* Linn. The variance observed in the result might be due to the harvesting conditions on which the plant is harvested along with environmental factors (Nordeide *et al.*, 1996; Kutbay *et al.*, 2001).The findings of the pharmacognostic study of the leaf/leaf powders of *M. oleifera* and *C. dactylon* analysed in our study were complying with the reports of Christi *et al*. (2021) and Dhanapal *et al.* (2017), respectively.

5. Conclusion

It can be concluded that the studies on physicochemical parameters can provide vital information about the quality of these plant materials for future investigations.Very few reports on the pharmacognostic study of the leaf/leaf powders of the plants were found; hence, our present study would be helpful in authentication of these plant species.

It would also help for the monographs of the plant for maximum therapeutic benefits. This study will also be beneficial in detecting the adulterants from the pure raw material of the plants under study. More pharmacological studies should be conducted with latest techniques to discover the medicinal potential of the plants.

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Authors' contribution

Both the authors of this paper equally contributed towards the experimental work as well as the writing of this paper.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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