International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 18-04-2019; Accepted: 22-05-2019
www.botanyjournals.com
Volume 4; Issue 4; July 2019; Page No. 90-99

Identity profile of *Moringa oleifera* Lam. Flower

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Abstract
Murungai poo, the flowers of *Moringa oleifera* Lam. are highly used in various traditional medical systems. The flowers contain bioactive phytochemicals and possesses promising antibacterial, antifungal, anti-larval, antioxidant, anti-inflammatory and anticancer properties. The authenticated flowers of *M. oleifera* were subjected to detailed macro microscopic and quantitative profiling, physico-chemical analysis, phytochemical screening and HPTLC. The flowers are zygomorphic. TS through pedicel was circular in outline, shows outer epidermis, middle cortex having abundant starch grains and inner pith region; TS through calyx and corolla showed numerous trichomes and mesophyll tissues but rosette crystals were present only in calyx. The powdered drug contained thick walled trichomes, parenchyma cells, vessels, pollen grains, rosette crystals and starch grains. The phytochemical screening gave substantial values of different parameters of the floral drug. The plants parts were subjected to TLC and HPTLC studies with CAMAG TLC and diagonostic peaks were recorded under UV 254 nm, 366 nm and 575 nm. This detailed pharmacognostic characterization will prove to be an exhaustive record for future reference and research on this herbal drug.

Keywords: Herbal monograph, HPTLC, *murungai poo*, quantitative microscopy, quality control, standardization

1. Introduction
Moringa oleifera Lam. commonly known as a Miracle tree owing to its immense medicinal properties belongs to the monogeneric family Moringaceae. *M. oleifera* and *M. concanensis* are the only two species of this genus recorded in India. This family has members with typically soft-wooded, deciduous trees [1, 2]. *M. oleifera* known as *Murungai* in Tamil is a small to medium sized tree cultivated all over the plains of India. It has now become widely known as a multi-purpose tree as it is grown for its nutritious pods, edible leaves and flowers in addition to the beneficiary properties as a source of food, medicine, cosmetic oil, forage for livestock and as a water coagulant [3].

Nearly all part of *M. oleifera* is used as medicine. Traditionally, the plant is used as antiseptic, anti-cancer, antispasmodic, anti-tubercular, anti-fertility, cardiac circulatorty tonic; fresh roots are used as antilithic, diuretic expectorant and stimulant. Bark is abortifacient, antifungal and antibacterial while the gum is bland and mucilaginous. Flowers are cholagogue, stimulant, tonic and diuretic and useful to increase the flow of bile. Seeds are acrid and stimulant [4].

The plants contains 4-hydroxy mullein, vanillin, mosinginine, bayrenol, indole acetic acid, indolacetonitrile, benzylisothiocynate, pterygospernin, carotene, flavanoids, polysaccharides, protein components, various essential amino acids, minerals and vitamins, fatty acids and spirochin [5]. *M. oleifera* leaf is a natural anthelminthic, antibiotic, detoxifier, outstanding immune builder used in some countries for the treatment of malnutrition and malaria [6]. Proximate analysis found that percentage of dry weight of proteins, ash, lipids, dietary fibre and nonstructural carbohydrate suggest a comparable nutritional profile for leaves and flowers [7]. Total antioxidant content of flower is also found higher than other plant parts [8]. The vitamin C content in flowers was found to be in highest when compared to other parts [9]. A number of qualitative analysis of various flower extracts confirmed the presence of saponins, tannins, alkaloids, flavonoids, steroids, glycosides, terpenoids and phenols [10, 11, 12, 13, 14]. According to Fuglie 2000 [15], *Moringa* is used in alley cropping, biogas,

fertilizer, nutrient, gum, honey and sugar from flower nectar and for medicine.

Murungai poo is the dried flower of *Moringa oleifera* used as cure for inflammations and muscle diseases [16]. In Siddha flower of *M. oleifera* is used in the preparation of *Panca cuta meluku* which is administered for curing cough, chest diseases, chronic bronchitis, urticaria caused by beetle sting, syphilitic ulcer, etc. [17]. An important treatise on Ayurveda depicts *M. oleifera* as Drusti Pathya meaning wholesome food for eyes [18].

Growing scientific evidence supports that this plant has medicinal benefits and its extracts could possibly be used as pharmacological interventions in various diseases [19]. Due to the proven potential as a nutraceutical investigations have been mainly concentrated on leaves and thus *Moringa* flowers received a little attention of researchers in spite of its significant nutritional and traditional healing properties [20]. Thus the current study was taken up for complete morpho-anatomical and chemical identity characterization of flower part of *M. oleifera*.

2. Materials and Methods

2.1 Collection and Identification of samples

The flowering twigs were collected from Siddha medical college campus Arumbakkam and authenticated with the help of Flora [21] at the department of Pharmacognosy, SCRI Chennai. The flowers were shade dried and packed in sealed covers for further studies.

2.2 Pharmacognostical Evaluation

2.2.1 Macroscopic characterization

The macroscopy of flowers were documented by Nikon COOLPIX5400 digital camera. Dissected floral parts were studied using Zeiss Stereo Discovery V.8 attached with Zeiss AxioCam ERc5s digital camera to reveal the morphology of the flower parts. The colour, odor and taste were also recorded [22].

2.2.2 Microscopic characterization

For microscopy all flower parts were hand cut into transverse sections using sharp platinum blade, stained with safranine and photographed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX5400 digital camera under bright field light. Magnifications were indicated by the scale-bars. Rest of the flowers were dried, powdered, passed through sieve no. 60, and preserved in air-tight containers for powder microscopy. The powder was mounted in glycerine on a clean microscopic slide, observed under Nikon ECLIPSE E200 trinocular microscope magnified to 400X and diagnostic characters were photographed. Quantitative microscopy of both sepal and petals was done by peeling epidermis and boiling about 20 minutes with 10% NaOH solution, the epidermii were peeled under distilled water, stained with safranine to count the epidermal cells, trichomes, vein islets and vein terminations in calyx and corolla by Camera lucida (Parco prism type) technique [23].

2.2.3 Physico-chemical analysis

The physico-chemical parameters like moisture content, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive and pH were determined according to the method mentioned in Siddha Pharmacopoeia [22].

2.2.4 High Performance Thin Layer Chromatography Sample Preparation

1 g of the sample was taken in a conical flask and 10 ml of ethanol was added, boiled for few minutes, cooled, filtered and then concentrated to 2 ml.

2.3 Mobile Phase

The mobile phase used was toluene: ethyl acetate: acetic acid (4:3:0.5 v/v/v).

2.4 Methodology

6 and 10 µl of the sample was applied on silica gel (60 F_{254}) pre-coated TLC plate using Camag ATS4 applicator. The plate was developed in a previously saturated twin trough chamber (CAMAG) of 10 cm × 10 cm size with the prepared mobile phase. The plate was developed up to 90 mm from the bottom. After development, the plate was photo documented using CAMAG TLC Visualizer under λ254 nm and λ366 nm. Then the plate was scanned using CAMAG Scanner 4 at λ254 nm (D2 lamp, Absorption mode) and λ366 nm (Hg lamp, Fluorescence mode) respectively and finger print profiles of the sample were documented. Subsequently the plate was dipped in vanillin sulphuric acid reagent followed by heating at 130ºC till development of the colored spots. The plate was then photo documented in white light using CAMAG TLC Visualizer and scanned at λ520 nm (W light, Absorption mode).

3. Results and Discussion

3.1 Macroscopy

Flowers are attached to terminal or axillary panicles; each flower is subtended by a bract with two bracteoles; sepals in quincuncial aestivation. The apical part of each petal appears elongated. The arrangement of petals is characteristic of the species and the plane of symmetry of the flower is transversal; floral zygomorphy is pronounced as the larger, posterior petal remains erect, while the others are reflexed together with the sepals; the front petals bear short hairs on their ventral side.

The flower is creamy white colored, irregular, bisexual in axillary panicles and measure 2.7 to 3.2 cm up to pedicel. Calyx cup-shaped and lobed with 5 unequal petaloid segments measuring 1.8 cm, pubescent outside; petals 5, unequal obovate to oblong and the lowest petal is the largest; measuring 1.4 x 0.6 cm; stamens 5 opposite to petals with 5 alternating staminodes, filaments free, anthers monothecous, dorsifixed; ovary stipitate; style slender, tubular; stigma perforated; ovules many, biseriate ovules on parietal placentation (Figure 1). Flowers have a peculiar sweet odour and taste is somewhat acrid turning to sweet.

3.2 Microscopy

Floral parts like pedicel calyx, corolla and gynoecium were subjected to histological studies.

3.2.1 Pedicel

TS of pedicel is circular in outline with outer epidermis, middle cortex and inner pith region; epidermis is covered by cuticle and bears numerous unicellular covering trichomes; epidermis is followed by 1 to 2 layers of isodiametric cells of hypodermis; cortex is made up of 2 to 4 layers of chlorenchyma cells followed by 4 to 8 layers of parenchyma cells; abundant starch grains are distributed in the cortical

cells; vascular ring shows patches of 20 to 22 vascular bundles formed by xylem and phloem elements present inner to the cortex; pith is made up of parenchyma cells (Figure 2).

3.2.2 Sepal
TS of sepal shows a single layer of both outer and inner epidermis consisting of isodiametric cells with thick cuticle, both epidermii have several unicellular trichomes; mesophyll composed of 18 to 20 layers of parenchyma cells filled with numerous starch grains, rosette crystals are scattered in the mesophyll region, oil globules are present in these cells; vascular tissue formed of xylem and phloem elements (Figure 3).

3.2.3 Petal
TS of petal showed both outer and inner epidermis covered with cuticle, and bears unicellular trichomes; loosely arranged parenchyma cells forms the mesophyll tissue; a row of small vascular bundles are present at the center portion formed of normal elements (Figure 4).

3.2.4 Anther
The TS of anther showed distinct layers of anther walls comprised of epidermis and endothecium, pollen sacs containing a huge amount of tricolpate pollen grains is present (Figure 5).

3.2.5 Ovary
TS of ovary shows circular outline with ridges and furrows. The single layered epidermis bears abundant unicellular trichomes. A ring of 9 to 10 vascular bundles are present in the parenchymatous cortical region. A pair of three ovules on parietal placentation can be seen inside the locule, intra-ovular trichomes also present between the ovules. The ovules are tricarpellary, apocarpous and campylotropous in nature (Figure 6).

3.3 Quantitative Microscopy
The calyx and corolla were subjected to quantitative microscopy. Dense unicellular trichomes were observed on both the surfaces of calyx and corolla. Epidermal number and stomatal density were similar but variations were observed in the number of trichomes with petal bearing greater number with respect to sepal (Figure 7, Table 1).

3.4 Powder microscopy
The powder was pale brown in colour with a characteristic smell and no particular taste. Epidermal cells with stomata, unicellular thick walled trichomes with blunt ends, fragments of parenchyma cells, palisade cells, cells of anther, oil globules, annular and spiral vessels, thin walled fibres with wide lumen, tricolpate pollen grains, rosette crystals and starch grains (Figure 8) were observed in powder.

3.5 Physico-chemical analysis
The loss of moisture was found to be 0.118%. The total inorganic was estimated to be 0.069 %. The percentage of water was calculated to be 0.053. The acid insoluble ash was found to be 0.0035 %. The water and ethanol soluble extractive were estimated as 33.35 & 17.6 % respectively. The acidic pH of 4.43 was recorded for the drug (Table 2).

3.6 TLC/HPTLC
TLC photo documentation revealed presence of phyto - constituents with different R_f values (Table 3). Densitometric scan of the plates showed diagnostic peaks under UV 254 nm, 366 nm and 575 nm post derivatization. A total of 6 bands were present each at 254 nm and 366 nm while 11 spots were visible at 575 nm (Figure 9).

Detailed macro-microscopic studies of *Moringa oleifera* flowers have shown many interesting characteristics. In most pentamerous flowers the zygomorphic symmetry line crosses the first or third sepal more or less in a median line [24]. But in *M. olifera*, an irregular development is seen with strongly apparent the floral zygomorphy. Each anther is laterally inserted and encloses the filament. Abaxially a groove is visible on each carpel. The presence of cup shaped calyx formed of 5 unequal petalloid segments arranged in imbricate aestivation, presence of five antesepalous staminode, monothecous anthers with hairy filaments are in accordance to the earlier studies carried out by Dutt *et al*., 1978 [25].

The studies conducted by Puri, 1942 [26] and Periasamy and Indira, 1986 [27] described the placentation to be laminal which was confirmed in the current study as a trimerous tricarpellary gynoecium with deviating placentation. This shifting of placentation from marginal to laminal position supports the classical interpretation of Dutt *et al*., 1978 [25], Cronquist, 1981 [28] and Periasamy and Indira, 1986 [27].

Endress and Stumpf, 1990 [29] described the anthers of *M. oleifera*. In the present study monothecous twisted anthers were observed. The ovary of *M. oleifera* is semi-inferior and remains free from receptacular tissue. The semi-inferior position of the ovary of *M. olifera* is caused by zonal growth below the perianth and stamens [30]. A hole like stigmatic aperture is present in *M. oleifera*. This is rare in eudicotyledons but has been observed in some Mimosaceae by Endress, 1994 [24] and in Caesalpiniaceae by Kantz and Tucker, 1994 [31]. In *M. oleifera* a spacious ovarial cavity is present and a large number of trichomes too which may functionally resemble obturators and facilitate the growth of pollen tubes. According to Dickison, 1993 [32] the occurrence of intraovarian trichomes is not common in Angiosperms.

Any matter other than the described parts of the drug is to be considered as foreign matter, any raw drug must be made free from foreign matter before any physico-chemical analysis is done Moisture leads to microbial growth which decreases the shelf life of drugs by deteriorating it. Total ash, an indicative of the total inorganic composition of the drug; water soluble ash indicative of the amount of ash which is readily soluble in water; loss on drying indicative of the moisture and volatile matter content in the sample; water soluble and alcohol soluble extractive values, indicative of polar compounds were determined. The solvent used for the extraction has to dissolve appreciable quantities of substances; likewise different solvents were used to extract these chemical constituents. The extract obtained by percolating coarse powder is indicative of approximate quantity of their chemical constituents.

TLC chromatograms of ethanol extract of *M. oleifera* flowers revealed 6 bands with R_f 0.01, 0.05, 0.15, 0.40, 0.52 and 0.61 (green) under short UV; 6 spots with R_f 0.02 (blue), 0.04 (ash), 0.60 and 0.95 (red), 0.89 (sky blue) 0.92 (pink) under long UV; 10 spots with R_f 0.06, 0.34, 0.82,

0.85 (blue), 0.17 and 0.43 (yellow), 0.60 and 0.65 (ash), 0.73 (violet) and 0.97 (dark blue) under white light (post derivatization) (Figure 9).

HPTLC finger printing is an effective technique of screening herbal raw drugs for authenticity and quality. In the HPTLC denistometric scan the fingerprint profile of ethanol extract under λ254 nm revealed that the major peak is R_f 0.94 with an area of 26.70% followed by the peaks at R_f 0.66 (23.41 %) and 10 more minor peaks; under λ366 nm, the major peak appeared at R_f 0.88 with an area 39.29 %, followed by the second major peak at R_f 0.94 with an area 22.69% along with 10 more peaks; under white light after derivatization, the peak at R_f 0.97 with an area 37.15 %came out as the major followed by the peak at R_f0.81 (26.80%) along with 11 more minor peaks.

All these pharmacopoeial parameter helps to determine the quality and purity of the important herbal drugs.

4. Tables and Figures

Table 1: Quantitative microscopy of *Moringa oleifera* flower

Parameters	Petal		Sepal	
	Upper (/mm^2)	Lower (/mm^2)	Upper (/mm^2)	Lower (/mm^2)
Epidermal number	230-300	200-350	158-200	150-290
Trichome number	153 - 180	160 - 185	100 -120	140 - 165
Stomatal number	0 -1	0 – 1	0 - 1	0 - 1

Table 2: Physico-chemical constants of *Moringa oleifera* flowers

Parameter	Mean ± SD
Loss on drying	0.118 ± 0.002
Total Ash	0.069 ± 0.001
Water soluble Ash	0.053 ± 0.004
Acid insoluble Ash	0.0035 ± 0.001
Water soluble extractives	33.35 ± 0.27
Alcohol soluble extractives	17.6 ± 0.23
pH	4.43 ± 0

Table 3: R_f values and color of spots of ethanolic extracts of *Moringa oleifera* flowers

Short 254 nm		Long 366 nm		White light 575 nm	
Color	R_f value	Color	R_f value	Color	R_f value
Green	0.01	Blue	0.02	-	-
Green	0.05	Ash	0.04	Blue	0.06
Green	0.15	-	-	-	-
-	-	-	-	Yellow	0.17
-	-	-	-	Blue	0.34
Green	0.40	-	-	-	-
-	-	-	-	Yellow	0.43
Green	0.52	-	-	Pink	0.51
Green	0.61	Pink	0.60	Ash	0.60
-	-	-	-	Ash	0.65
-	-	-	-	Violet	0.73
-	-	-	-	Blue	0.82
-	-	Sky Blue	0.89	Blue	0.85
-	-	Pink	0.92	-	-
-	-	Pink	0.95	-	-
-	-	-	-	Dark Blue	0.97

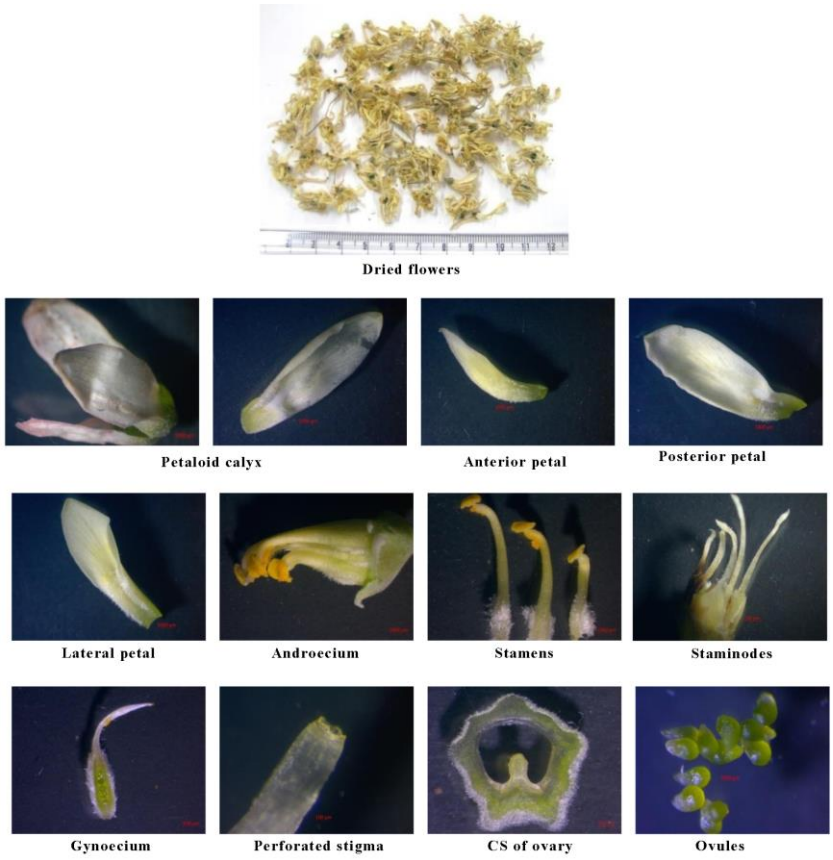


Fig 1: Macroscopy of *Moringa oleifera* flower

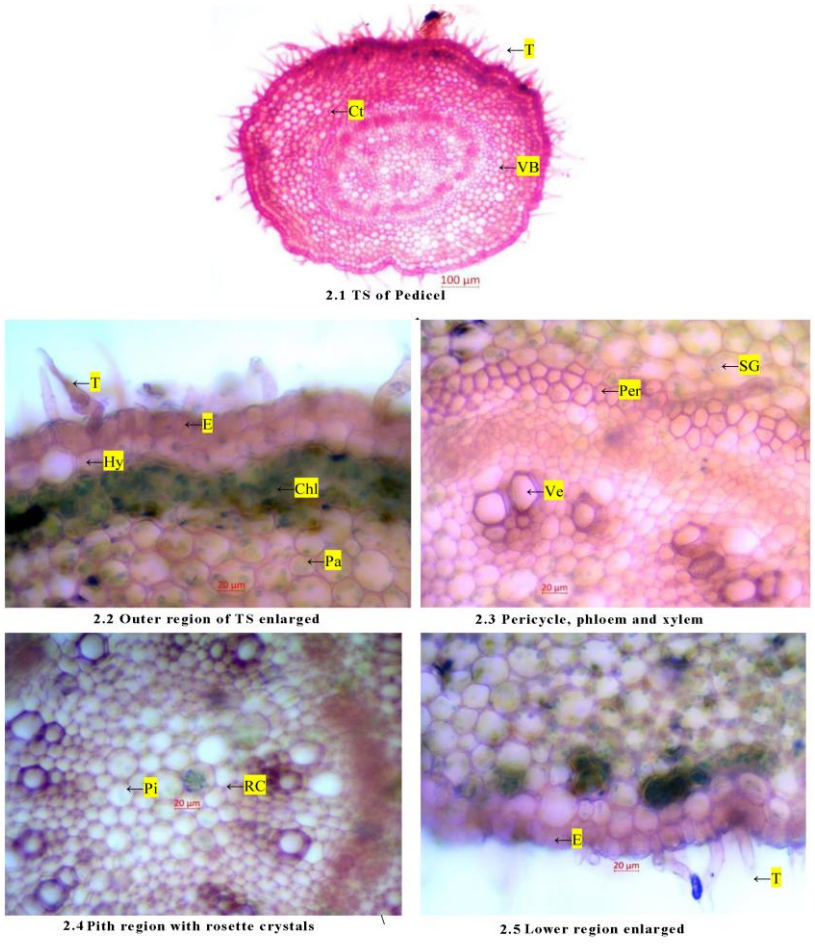


Fig 2: Microscopy of pedicel of *Moringa oleifera* flower

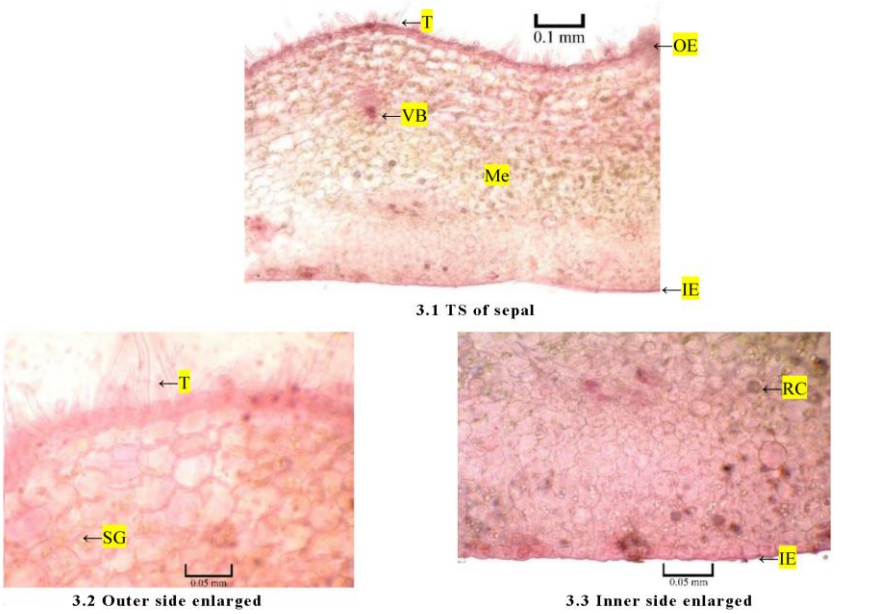


Fig 3: Microscoy of sepal of *Moringa oleifera* flower

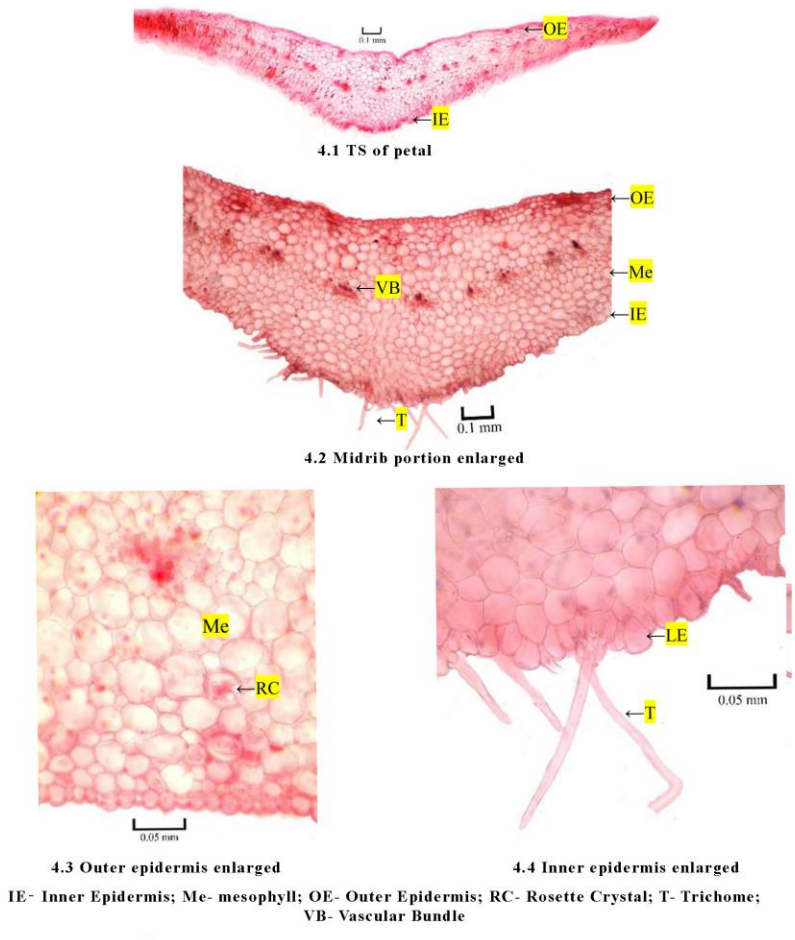
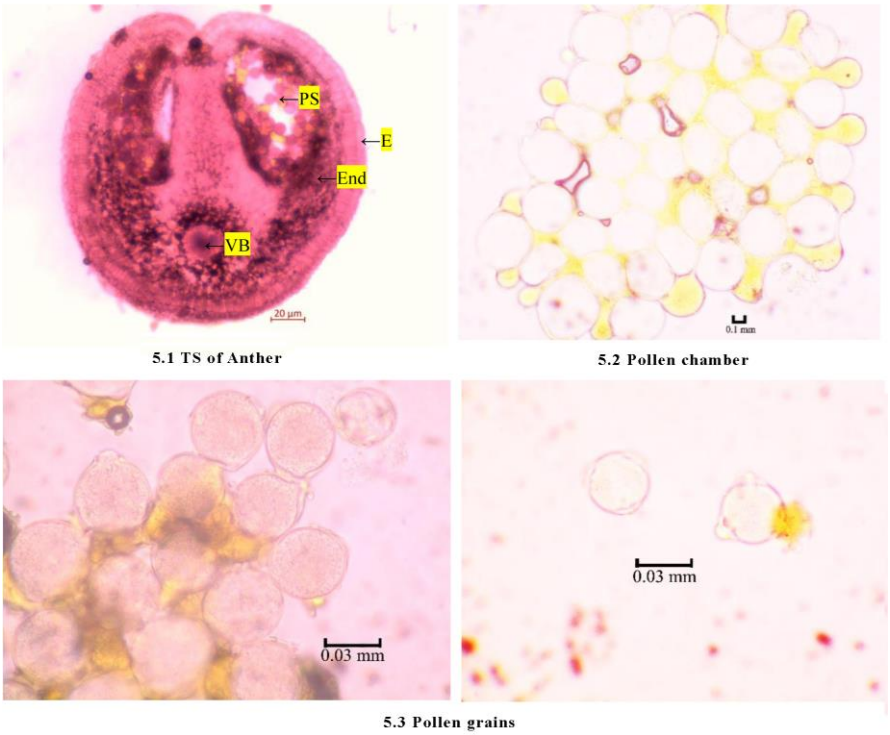


Fig 4: Microscopy of petal of *Moringa oleifera* flower



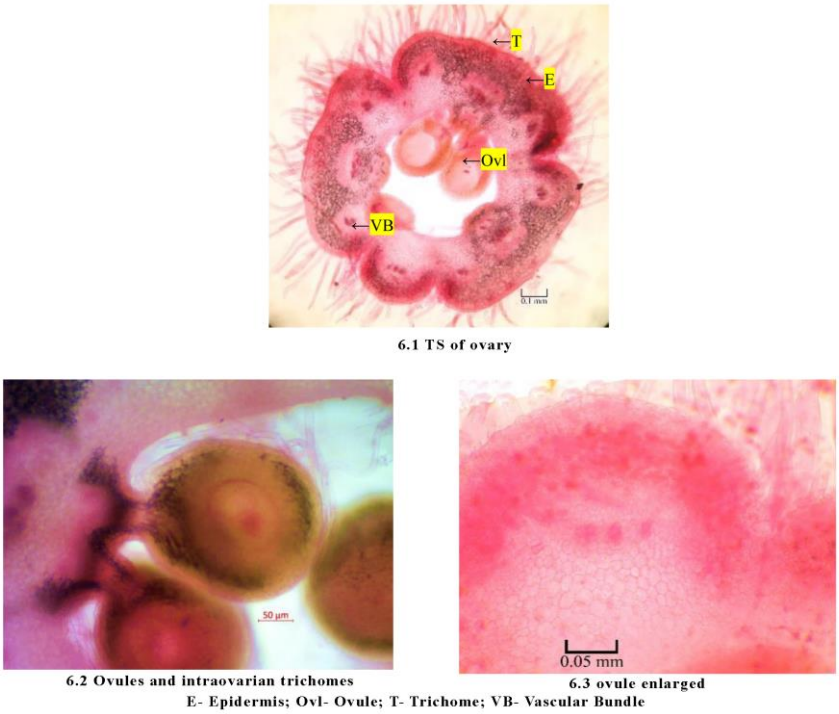
5.1 TS of Anther

5.2 Pollen chamber

5.3 Pollen grains

E- Epidermis; End- Endothecium; PS- Pollen Sac; VB- Vascular Bundle

Fig 5: TS of Anther and pollen grains of *Moringa oleifera* flower



6.1 TS of ovary

6.2 Ovules and intraovarian trichomes

6.3 ovule enlarged

E- Epidermis; Ovl- Ovule; T- Trichome; VB- Vascular Bundle

Fig 6: Microscopy of ovary of *Moringa oleifera* flower

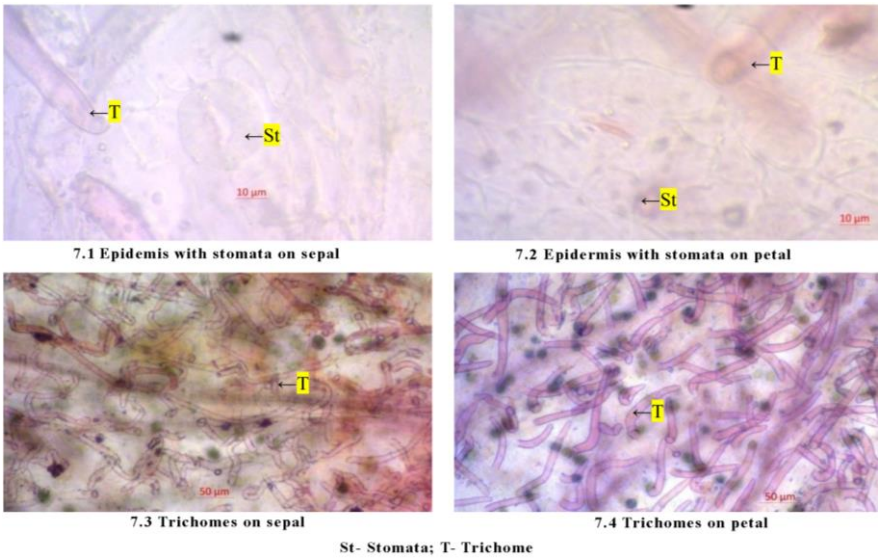


Fig 7: Quantitative microscopy of *Moringa oleifera* flower

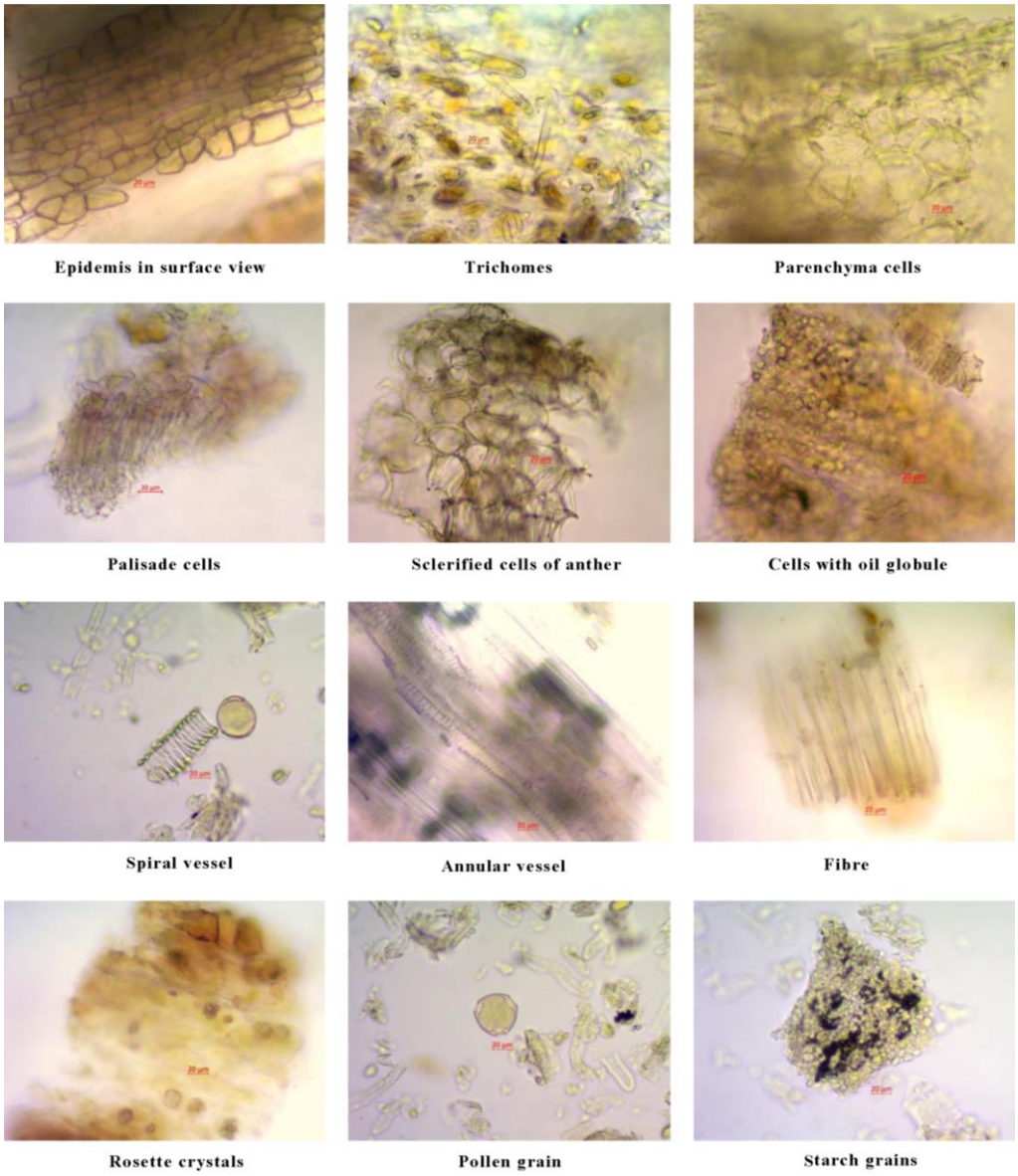


Fig 8: Microscopy of powder of *Moringa oleifera* flower

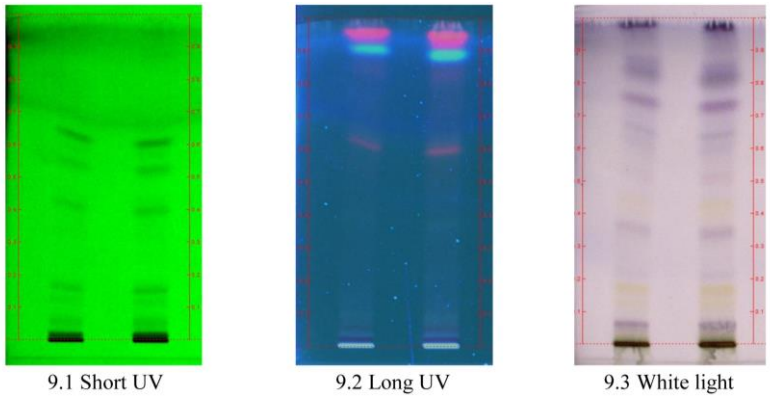
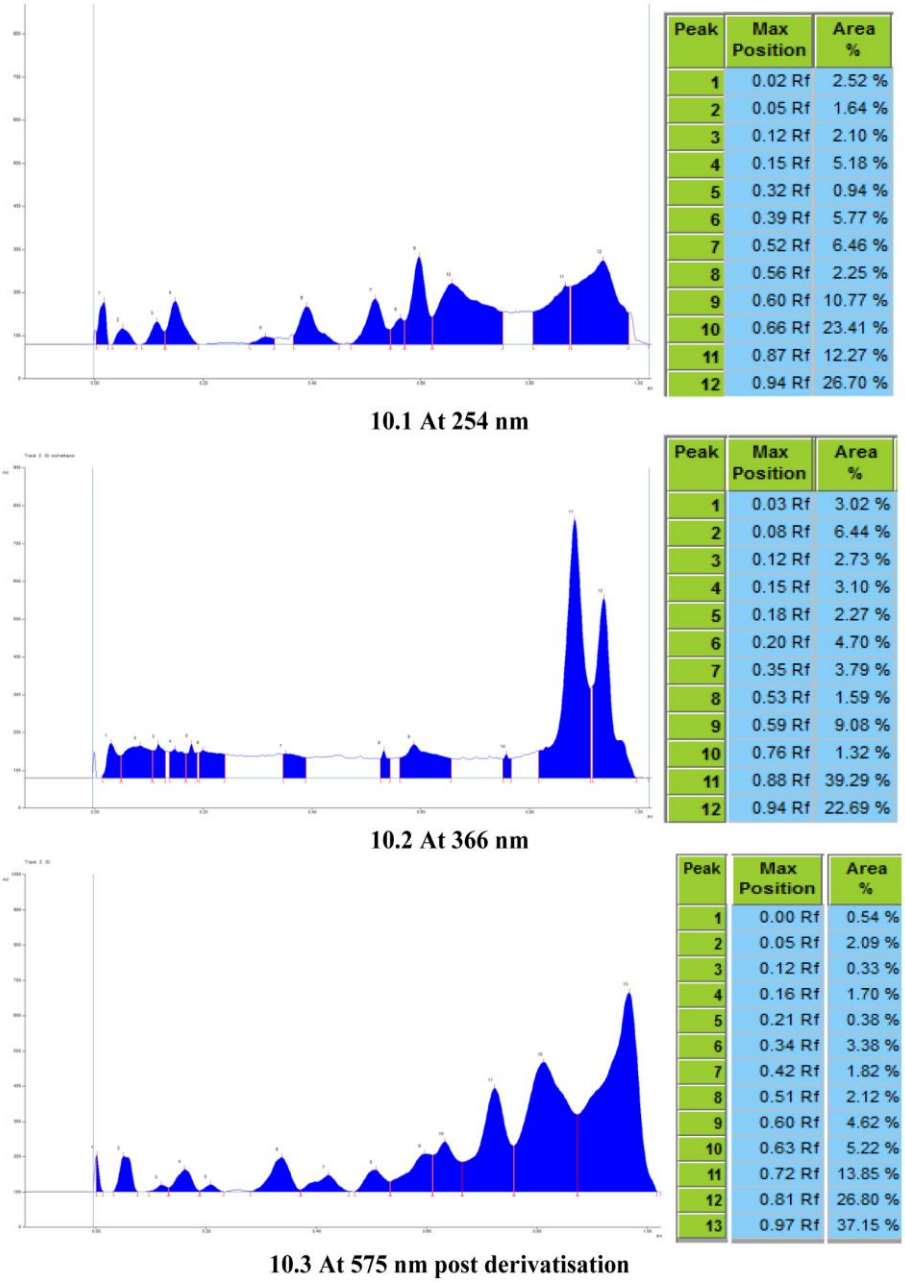


Fig 9: TLC-photodocumentation of ethanolic extract of *Moringa oleifera* flower



Peak	Max Position	Area %
1	0.02 Rf	2.52 %
2	0.05 Rf	1.64 %
3	0.12 Rf	2.10 %
4	0.15 Rf	5.18 %
5	0.32 Rf	0.94 %
6	0.39 Rf	5.77 %
7	0.52 Rf	6.46 %
8	0.56 Rf	2.25 %
9	0.60 Rf	10.77 %
10	0.66 Rf	23.41 %
11	0.87 Rf	12.27 %
12	0.94 Rf	26.70 %

10.1 At 254 nm

Peak	Max Position	Area %
1	0.03 Rf	3.02 %
2	0.08 Rf	6.44 %
3	0.12 Rf	2.73 %
4	0.15 Rf	3.10 %
5	0.18 Rf	2.27 %
6	0.20 Rf	4.70 %
7	0.35 Rf	3.79 %
8	0.53 Rf	1.59 %
9	0.59 Rf	9.08 %
10	0.76 Rf	1.32 %
11	0.88 Rf	39.29 %
12	0.94 Rf	22.69 %

10.2 At 366 nm

Peak	Max Position	Area %
1	0.00 Rf	0.54 %
2	0.05 Rf	2.09 %
3	0.12 Rf	0.33 %
4	0.16 Rf	1.70 %
5	0.21 Rf	0.38 %
6	0.34 Rf	3.38 %
7	0.42 Rf	1.82 %
8	0.51 Rf	2.12 %
9	0.60 Rf	4.62 %
10	0.63 Rf	5.22 %
11	0.72 Rf	13.85 %
12	0.81 Rf	26.80 %
13	0.97 Rf	37.15 %

10.3 At 575 nm post derivatisation

Fig 10: Densitometric scan of ethanolic extract of *Moringa oleifera* flower

5. Conclusions

Thus the present study ascertains the macro microscopic detailing of *M. oleifera* flowers together with the development of simple, specific, accurate and reproducible TLC method for the qualitative identification of the various components makes this floral drug a considerably interesting one and can lead to the development of new drugs in the future. So the present study which

reinvestigated the pharmacognostic details of *M. oleifera* can prove to be a worthwhile catalogue for the authentication and identification of this valuable drug which finds immense potential in the drug industry.

6. Acknowledgments
The authors extend their heartfelt thanks to Director General, CCRS and Assistant Director in Charge for their support.

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