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Original Research Article (Experimental)

Pharmacognostic characterization of *Myrica esculenta* leaves

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ABSTRACT

Background: *Myrica esculenta* (Family: Myricaceae) commonly known as *Kaiphala* or *Katphala* is a widely used medicinal plant in Ayurveda. In spite of its numerous medicinal attributes, no published work is available till date on pharmacognostical characterization and HPTLC analysis of its leaves.
Objectives: To investigate the pharmacognostical, physicochemical, and HPTLC profiles of *M. esculenta* leaves.
Materials and methods: The measures taken for pharmacognostical characterization were organoleptic study, macroscopy, microscopy, powder microscopy, leaf constant, fluorescence analysis, preliminary phytochemical screening and HPTLC spectra profile.
Results: Organoleptic and macroscopic studies found that leaves are lancoelate, thin, spirally arranged, dark green in color, with an astringent taste and acute apex. In transverse section, cuticularised epidermis having polygonal cells were found. Mesophyll cells were differentiated into single layered palisade cells on each surface and 2–3 layered spongy parenchyma, unicellular and uniseriate hollow trichomes, anomocytic stomata and bowl shaped vascular bundle in mid rib portion containing xylem and phloem tissues. Alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds and tannins were found present. Analysis on the leaf constants, powder microscopy, fluorescence characteristics and physical parameters resulted a valuable data to establish standards for the plant. HPTLC profile provides number of constituents present in the extracts with their respective Retention Factor (R_f).
Conclusion: Present report on pharmacognostical characterization and HPTLC analysis of *M. esculenta* leaves provides a vital diagnostic tool for identification, authentication and development of quality parameters of the species. Data obtained by present study may be considered as standard for future studies.

1. Introduction

India has a wide range of traditional medicines from ancient time, based on various medical systems like Unani, Siddha, Homeopathy and mainly Ayurveda. Plants are used as medicine to maintain human health from ages [1] and are also major natural sources of medicinal compounds in current pharmacopoeias [2]. Therefore, there is a need to evaluate phytoconstituents obtained from traditional medicines, based on various phytochemical screening, pharmacological and analytical methods [3]. The very first step in this direction is the herb characterization or detailed pharmacognostic evaluation that gives external appearance, microscopy and physical characteristic of the crude drug [4].

Myrica esculenta Buch-Ham (Family – Myricaceae) Syn., *Myrica nagi* Hook., commonly known as *Kaiphala* or *Katphala* is an evergreen dioecous tree, found mainly in Punjab, Uttarakhand (Garhwal), Kumaun hills, Khasiya mountain and Sylhet in India at elevations of upto 2100 m. Some different varieties are also found in neighboring countries like Nepal, China etc. It is a shade giving, medium sized tree, having a big crown on it. The bark extract has already been reported to contain steroids, reducing sugars, tannins, glycosides, saponins and volatile oils. The hexane extract of the *Katphala* roots revealed the presence of friedelin and β–sitosterol [5]. *Katphala* is widely used in Indian system of medicine and various parts of this botanical are used in conditions like *Gulma* (abdominal lump), *Jvara* (fever), *Arsha* (piles), *Grahani* (irregular bowel function), *Pandu roga* (anemia), *Hrillasa* (nausea), *Mukha roga* (oral disorders), *Kasa* (cough), *Shvasa* (dyspnea), *Agnimandhya* (indigestion), *Aruchi* (anorexia) and *Kantharoga* (ears, nose, and

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throat disorders) [6]. Stem bark is extensively explored for various pharmacological roles likes free radical-scavenging [7], antioxidant [7,8], anti-diabetic [9], anxiolytic [10], antibacterial [11], anti-helmintic [12], anti-allergic [13], anti-inflammatory, antimicrobial [14], mast cell stabilizing [15] and anti-asthmatic [16]. The plant has tremendous therapeutic potential with every part of the plant being used medicinally, including leaves.

Earlier reports established pharmacognostical and phytochemical profiles of *M. esculenta* stem [17,18]. In spite of its numerous medicinal attributes, no published work is available till date on pharmacognostical characterization and HPTLC analysis of its leaves. Considering this, the present study was undertaken to develop a quality standard for leaves of this plant. In the present study, investigations were attempted to record the organoleptic characters, macroscopy, microscopy, quantitative values such as physical parameters, phytochemical screening and chromatographic profiles of *M. esculenta* leaves. This would be a milestone in assessing the quality of the crude drug for further development.

2. Materials and methods

2.1. Collection and authentication of plant materials

Leaves of *M. esculenta* were collected from outskirts of Chail Chowk, Mandi, Himachal Pradesh (H.P.) in November 2016. The plant was identified, authenticated and certified (AGI/2015/1220) by Head, Department of Botany, Abhilashi Group of Institutions, Mandi, Himachal Pradesh.

2.2. Chemicals

Toluene, safranin, chloral hydrate, ethyl acetate, methanol, and all other analytical grade chemicals were purchased from E. Merck Limited India and HiMedia Laboratories, Mumbai, India.

2.3. Organoleptic evaluation

Leaves of *M. esculenta* were evaluated for their impact on various organs of sense for organoleptic properties. Its color, odor, size (length and width), taste and other diagnostic parameters was observed and noted [19].

2.4. Macroscopic analysis

Macroscopic features of the leaf viz. type of leaf, shape, apex, margin, lamina, base venation and texture were analyzed by standard method [19].

2.5. Microscopical analysis

2.5.1. Study of transverse section

The potato sandwiched specimens were sectioned with the help of a sharp blade. The obtained thin slide was stained by safranin dye (0.1% w/v solution in distilled water). Photographs of different magnification were taken with Nikon lab photo 2 microscope unit.

2.5.2. Powdered drug microscopy

The coarsely powdered leaf was mounted on a glass slide in glycerin and studied under a microscope. Photographs of different magnified cellular structures were taken with Nikon lab photo 2 microscope units.

2.5.3. Analysis of leaf constants

Different leaf constants viz. vein islet number, vein termination number, stomatal index, stomatal number, palisade ratio were analyzed using standard procedures [20].

2.6. Physiochemical analysis

Physiochemical parameters (ash value, moisture content, foreign matter, fluorescence analysis, extractive value) were determined [21,22].

2.7. Preliminary phytochemical screening

Various extracts of *M. esculenta* leaves were subjected to phytochemical analysis [23]. A series of identification tests were performed to detect presence of alkaloids, flavonoids, saponins, proteins and amino acids, fixed oils and fats, glycosides, tannins and steroids.

2.8. Preparation of extracts

Firstly, the plant leaves were washed with water to remove dirt and other foreign matters and were separated and shade dried. Dried leaves were then milled to coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of *M. esculenta* (500 g) were placed in the tube of Soxhlet apparatus in the form of thimble and kept on heating mental for 6 h for extraction using various solvents as ethyl acetate, methanol and water. The obtained extracts were filtered while hot and dried by evaporation using rotary vacuum evaporator and the final dried extracts samples were kept at low temperature in fridge for further study.

2.9. Development of chromatographic profile by HPTLC of various extracts of M. esculenta

CAMAG-HPTLC system having a sample applicator Linomat 5 was used to obtain HPTLC chromatograms using standard methods [24]. HPTLC profile of the various extracts of *M. esculenta* was developed using toluene:ethyl acetate (7:3) as mobile phase to confirm the occurrence of different phytoconstituents. The chromatographic development processes were performed in an air-conditioned room where the temperature was maintained at 22 °C and relative humidity was maintained at 55%. Ready to use silica gel-G precoated HPTLC aluminum plates were used for chromatographic separation. The extract (10 μL) was spotted as bands of 10 mm width with the help of the auto sampler fitted with a 100 μL Hamilton syringe. The solvent system was transferred to CAMAG Twin Trough plate development chamber lined with filter paper and pre-saturated with mobile phase (25 mL) for 30 min. The resulted plates were air dried and scanned. Developed spots on the places were scanned at the wavelength of 254 and 366 nm respectively. The retention factor (R_f) value for each spot found on plate was recorded.

3. Results

3.1. Organoleptic and microscopic characters

M. esculenta leaves are dorsiventral with a fairly prominent midrib and thin smooth lamina. They are of dark green color having leathery finish and a somewhat light green color on abaxial surface as compared to adaxial surface. The leaves are of yellowish green color in early ages which become darker with age. Leaves were odorless but having a slight bitter and astringent taste that may be due to presence of tannins.

Fig. 1. Macromorphological features of *M. esculenta*.

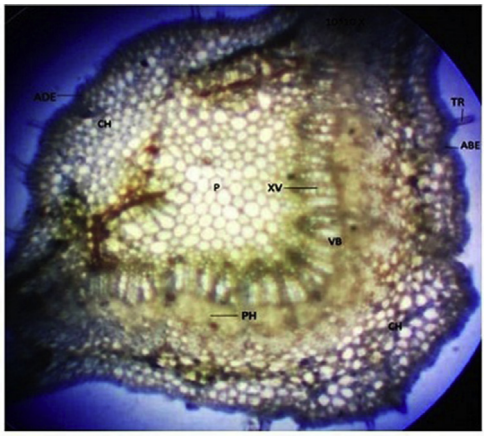
3.2. Macroscopic characters

The leaves are laceolate with an average petiole measuring 0.4–1.5 cm in length. The lamina of leaf is smooth at both the surfaces with entire margin and an acute apex. In some leaves ¼ part of the margin near to apex is serrate. The leaves were 5–10.2 cm long and 2–3.5 cm broad. Phyllotaxy of the leaves is spiral and venation is reticulate pinnate (Fig. 1).

3.3. Microscopic characters

3.3.1. Transverse sections of leave

Transverse section is showed in Figs. 2–4. The upper and lower epidermis consisted small single layered thick polygonal cells which appear wavy in outline, covered by a thin cuticle containing



TR: Trichomes; ADE: Adaxial Epidermis (Upper Epidermis); ABE: Abaxial Epidermis (Lower Epidermis); P: Parenchymatous Tissue; XV: Xylem Vessels; PH: Phloem Tissue; CH: Chollenchymatous Tissue and VB: Vascular Bundles

Fig. 2. Transverse section of leaf of *M. esculenta* viewed at 10*10X

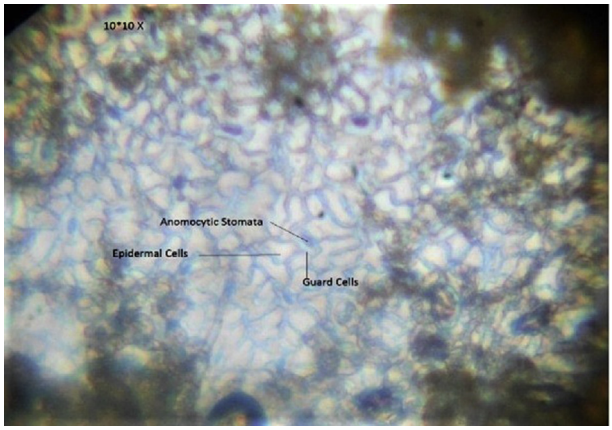


Fig. 3. Section of lower surface of *M. esculenta* leaf at 10*10 X view (stomata clearly depicted the structure of an anomocytic).

mucilage. Both epidermises are abundant in unicellular uniseriate hollow trichomes (Fig. 2). Lamina region is differentiated into regular, long, columnar cells of palisade tissue and 3–4 layers of spongy parenchyma which is extended upto midrib region (Fig. 4). There are 4-6 layers of collenchymatous cells on both sides in the midrib region after epidermis, and the rest are several layers of spherical spongy parenchymatous tissue extended from lamina. The vascular strand embedded in spongy parenchyma of midrib region consist of long compact row of thick walled xylem elements and small nests of phloem tissue, both of which form a bowl shape in the center (Fig. 2). The stomata were of anomocytic type on both of the surfaces. Guard cells are surrounded by limited subsidiary cells which are rarely differentiated from epidermal cells (Fig. 3). Abaxial surface (lower epidermis) contains more stomata as compared to adaxial surface.

3.3.2. Powdered drug microscopy

The powder was green in color, with no distinct odor and an astringent taste. It was easy flowable with a fine to coarse texture. Microscopic powder features revealed presence of crystals of calcium oxalate and starch granules; trichomes were found to be hollow unicellular and uniseriate; parts of epidermis in surface view showing straight-walled polygonal epidermal cells,

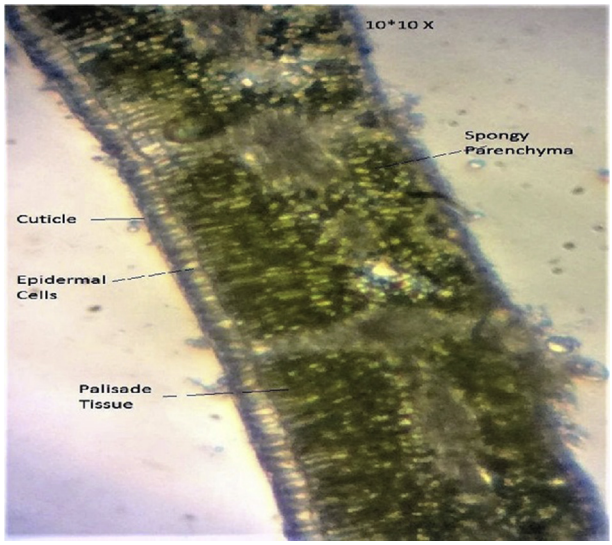


Fig. 4. Transverse section of leaf lamina of *M. esculenta*.

anomocytic stomata and spiral xylem vessels. The guard cells were shrinking elongating the stomata pore (Fig. 5).

3.3.3. Leaf constants

Anomocytic types of stomata were present on the both surfaces of leaves. The stomatal number on the adaxial and abaxial surface was found as 2.14 and 4.25, respectively. The stomatal indexes of adaxial and abaxial surface was found 16.67 and 17.33, respectively. The vein islet and vein termination were calculated as 9-11 and 13-15. The palisade ratio was found to be 2-4.

3.4. Physicochemical evaluation

The results of physicochemical parameters are summarized in Table 1. Sulphated ash value (2.41%) was found lower than the total ash value (2.83%). The acid insoluble ash was found to be 0.52% and water soluble ash value was 0.38%. On further studies it was concluded that the drug contain 8.72% moisture content, foaming index and swelling index were found to be nil, while foreign organic content was reported to be less than 1%. The extractive values for various solvents such as methanol, ethyl

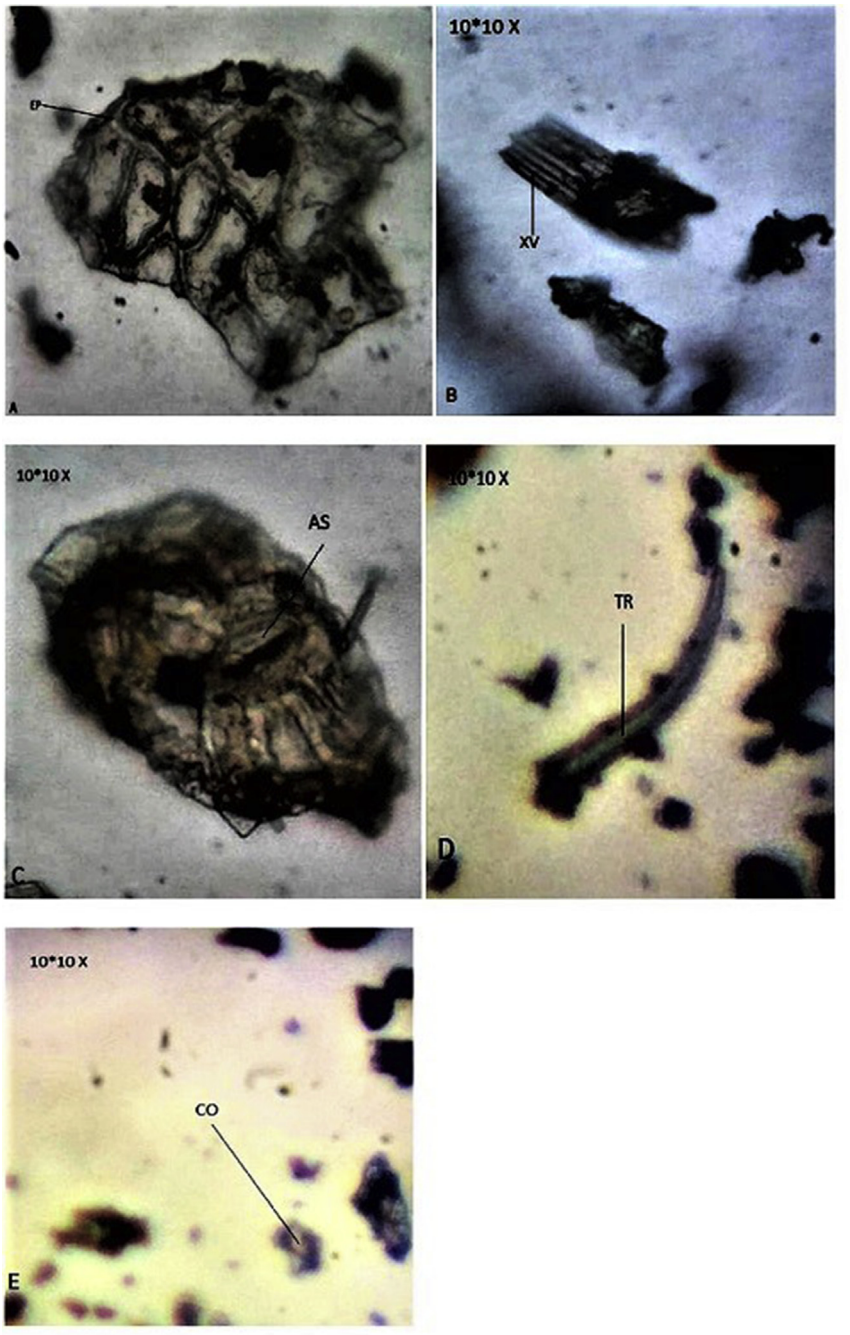


Fig. 5. Powdered characteristics of the leaf of *M. esculenta*.

Table 1
Physicochemical parameters of leaves of *M. esculenta*.

Physiochemical parameters		Results (%)
Ash value	Total ash	2.83
	Acid insoluble ash	0.52
	Water soluble ash	0.38
	Sulphated ash	2.41
Extractive value	Methanolic extract	38.51
	Ethyl acetate extract	21.2
	Aqueous extract	15.7
Moisture content		8.72
Foreign organic matter		Less that 1% (Presence of petiole stalks with leaves)
Foaming index		Nil
Swelling index		Nil

acetate and aqueous were found to be 21.2%, 15.7%, 8.72% respectively.

3.5. Preliminary phytochemical screening

Various phytochemical analysis tests supported that the extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides, recorded in Table 2. The aqueous extract was found to be negative for the presence of alkaloids as compared to methanolic and ethyl acetate extract.

3.6. Fluorescence analysis

The fluorescence characters of powdered drug impart a valuable role in the determination of quality and purity of the drug materials. The powdered drugs when subjected to ultraviolet light and visible light in the presence of various chemical reagents, exhibit characteristic fluorescence [20]. Fluorescence report of *M. esculenta* powdered leaf is tabulated in Table 3.

Table 2
Results of phytochemical screening of different extracts of *M. esculenta* leaves extracts.

Constituent	Leaves extracts		
	Aqueous extract	Ethyl acetate extract	Methanolic extract
Alkaloids	−ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve
Proteins & amino acids	−ve	−ve	−ve
Fixed oils & fats	−ve	−ve	−ve
Flavonoids	+ve	+ve	+ve
Phenolic compounds	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Saponins	−ve	−ve	−ve
Steroids	−ve	−ve	−ve

+ve- Present, −ve- Absent.

3.7. Development of chromatographic HPTLC profile of different extracts of M. esculenta leaves

Qualitative HPTLC of ethyl acetate extract, methanolic extract and aqueous extract was carried out by using toluene:ethyl acetate (7:3) solvent system to assure the presence of various phytochemicals. HPTLC chromatogram of ethyl acetate extract (Fig. 6) showed a total of 3 spots at different R_f value at 254 nm, whereas 7 spots were observed at 366 nm (Fig. 6). Methanolic extract showed 2 spots having different R_f values at 254 nm while 5 spots at 366 nm (Fig. 6). Least spots were observed in aqueous extract. There were a total of 2 spots at 254 nm and 3 spots at 366 nm having different R_f values (Fig. 6).

4. Discussion

M. esculenta is used extensively as an ancient traditional medicine for the treatment of wide range of diseases [6–16]. But there are no pharmacopeial standards for the correct identification and authentication of leaf of the plant. Thus, in the present study, the leaf part of *M. esculenta* was analyzed for its pharmacognostic features. *M. esculenta* is an evergreen tree having leaves of green color, lanceolate having a smooth, leathery lamina with acute apex [5]. The TS examination and powder microscopy showed a prominent dorsiventral leaf features with presence of calcium oxalate crystals. There are uniseriate hollow trichomes and anomocytic stomata were present on epidermis making these features as important diagnostic characters. Further leaf constant can also be used for identification of leaf and analyzing purity and adulterations.

Physicochemical evaluations provide various important parameters like moisture content, ash values and extractive values for various solvents. The ash values determined in the present study may be useful in stabilizing standards of purity and quality. The low total ash value indicate the low amount of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. The acid insoluble ash was very low that support the fact that a very small amount of the inorganic component is insoluble in acid and this is a diagnostic tool. The moisture content of the fresh leaves was 8.72% showing the leaf dried easily after plucking. The extractive values gives an idea about the nature of the chemical constituents present in the plant and is useful for the estimation of specific constituents soluble in that particular solvent used for the extraction as well as the determination of exhausted materials. The methanol and ethyl acetate soluble extractives were higher than that of water.

Table 3
Fluorescence characteristics of leaves extracts.

S. no.	Treatment	Visible light	UV Light	
			254 nm (Short wavelength)	366 nm (Long wavelength)
1	Leaf powder	Green	Green	Light Green
2	Leaf powder rubbed on filter paper	Light Green	Light Yellow	Light Green
3	Leaf powder + 1N NaOH	Reddish Brown	Black	Brown
4	Leaf powder + 1N HCl	Light Green	Light Yellow	Light Green
5	Leaf powder + 1N HNO_3	Light Yellowish Green	Light Yellow	Light Yellow
6	Leaf powder + 1N H_2SO_4	Light Green	Light Green	Light Green
7	Methanolic extract of Leaf powder	Dark Green	Black	Green
8	Ethyl acetate Extract of Leaf powder	Green	Light Green	Light Green
9	Aqueous extract of leaf powder	Greenish Brown	No fluorescence	Brown

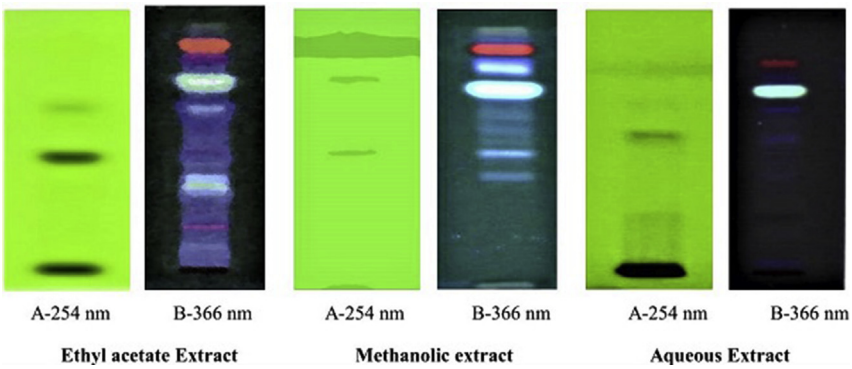


Fig. 6. Visualization of ethyl acetate, methanolic and aqueous extract of *M. esculenta* leaves at 254 and 366 nm.

Thus, these are a good choice as solvent for extraction of leaves of *M. esculenta*. The fluorescence study showed characteristic fluorescence under visible light and UV light (short and long wave length), and this may be useful in the detection of adulterants.

Preliminary phytochemical screening was done to check the presence of active constituents of the leaves. It reveals the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides. The HPTLC chromatogram of various extracts may be useful in the confirmation of presence of number of constituents along with the respective R_f value.

5. Study limitations and future directions

Limitation of present study is the HPTLC carried out is without use of standard/active compound. Since present report is focused mainly on pharmacognostic evaluation and preliminary phytochemical screening; here HPTLC was performed to confirm the number of phytoconstituents present in different extracts and to support the phytochemical screening. *M. esculenta* leaves are known to contain very important phytoconstituents, therefore by using standards, different constituents can be identified and even isolated in future studies. Data obtained by present study may be considered as standard for future studies.

6. Conclusion

Macro-microscopic studies revealed that leaves are lancoelate, thin, spirally arranged, dark green in color, astringent taste, acute apex, cuticularised epidermis having polygonal cells, single layered palisade mesophyll cells on each surface, 2–3 layered spongy parenchyma, unicellular and uniseriate hollow trichomes, anomocytic stomata and bowl shaped vascular bundle in mid rib portion. Alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds and tannins were found present. HPTLC provides number of constituents present in the extracts with their respective R_f value. Present pharmacognostical assay of *M. esculenta* leaves including data on leaf constants, powder microscopy, fluorescence characteristics and physical parameters provides a vital diagnostic tool for identification, authentication, and detection of adulterants of *M. esculenta* leaves, along with development of quality parameters of the species.

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Conflict of interest

None.

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