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Pharmacognostic, physicochemical and phytochemical investigation of *Grangea maderaspatana*

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ABSTRACT

Grangea maderaspatana is a popular Indian medicinal plant belonging to the Asteraceae family. This plant is commonly known as Madras Carpet. It has long been used in traditional Ayurvedic medicine for various diseases. Literature reveals that the phytoconstituents like steroids, terpenes, flavonoids, saponins, carbohydrates, phenolics and tannins are present in the plant. Pharmacognostical standards on *G. maderaspatana* are not yet available for correct identification of plant material and to ascertain its quality and purity. The present study was therefore undertaken to determine the required pharmacognostical standards according to the Pharmacopoeial guidelines for evaluation of the plant. The study included examination of morphological, microscopical, physicochemical properties, phytochemical analysis and HPTLC fingerprinting. The standardization parameters presented in this paper may be suggested to establish the authenticity of *Grangea maderaspatanaand* can also help to distinguish the drug from its other species. The pharmacognostic profile of the plant presented here will contribute in standardization viz., quality, purity and sample identification.

Keywords: *Grangea maderaspatana*, Asteraceae, Standardization.

INTRODUCTION

Grangea maderaspatana is a popular Indian medicinal plant belonging to the Asteraceae family. This plant is commonly known as Madras Carpet growing in sandy lands and waste places. It is reported to contain flavonoids, diterepenes, sesquiterpenoids, steroid, and essential oil. The herb is good for pain in the eyes and ears. The root is an appetizer; astringent to the bowels, diuretic, anthelmintic, emmenogogue, galactogogue, stimulant; useful in griping, in troubles of the chest and lungs, headache, paralysis, rheumatism in the knee joint, piles, pain in the muscles, diseases of the spleen and the liver, the troubles of the ear, the mouth and the nose; lessens perspiration (Unani). The plant is stomachic and uterine stimulant [1].

Figure 1: *G. maderaspatana* Plant

MATERIALS AND METHODS

Plant collection and Identification

The plant of *Grangea maderaspatana* was collected in the month of December from Saputara, Gujarat. Authentication was done by Taxonomist of the Botanical Survey of India, Jodhpur. A voucher specimen

(No BSI/AZRC/I.12012/Tech./2015-16/419) was deposited in the Herbarium of Botanical Survey of India, Jodhpur.

Macroscopic and microscopic investigation

The macroscopic features of *Grangea maderaspatana* leaf, stem and root was determined using prescribed method[2]. For microscopical examinations, free hand sections of the fresh leaf, stem and root were cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric acid and phloroglucinol. Photomicrographic images were taken by using Trino CXR camera.

Leaf constant

Leaf constants such as stomatal index, vein islet number, vein termination number and palisade ratio were studied according to standard methods [2].

Physicochemical analysis

Physicochemical analysis, such as extracting values, Loss on drying and ash values were performed according to the official methods [3, 4, 5].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures [6, 7].

HPTLC Fingerprinting

HPTLC fingerprinting of chloroform and methanol extracts of *G. maderaspatana*was performed for oleanolic acid and ursolicacid [8].

a) **Sample preparation:** Accurately weighed 20 mg of each extracts individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.
b) **Standard preparation:** Accurately weighed 10 mg of each standard individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Application of sample	Automatic device "CAMAG LINOMAT – 5"
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets (10 x 10 cm)
Application Volume	10 µL
Mobile Phase	Toluene: Ethyl acetate: Formic acid (8: 2: 0.1)
Development Mode	CAMAG TLC Twin Trough Chamber
Spray reagent	Anisaldehydesulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Visualization	@ 510 nm after derivatization

RESULTS

Macroscopic study

The leaves of *Grangea maderaspatana* are simple, alternate, sessile, Oblong-obovate, coarsely dentate, obtuse, reticulate, 3.5-7.5 cm long and 1.5-2.5 cm wide. They are dark green in color with characteristic odor and taste. The stem is Prostrate, ascending to erect, which is up to 55 cm tall, branched from the base. The stem is green colored with numerous hairs on the surface. Roots are primarily tap-rooted but form weak adventitious roots along the stolon nodes. They are white to brown color with characteristic odor and taste.

Flowers: The inflorescence is a terminal, truncate-spherical head, 6-10 mm in diameter, solitary or 2-3 together, yellow colored. The peduncle is 1-4 cm long. The flowers are all tubular and about 1.5 mm long.

Fruit: The fruit is pale brown and compressed. Achenes are cylindric, glandular, and about 2 millimeters long.

Microscopic study

Leaf: The transverse section of leaf of *Grangea maderaspatana* shows dorsiventral nature. The section is broadly divided in to lamina and midrib region. The lamina of leaf shows three distinct regions viz., upper epidermis, lower epidermis and mesophyll. The upper and lower epidermis is single layer of cells covered by cuticle. The mesophyll is differentiated in to palisade and spongy parenchyma. The palisade parenchyma are narrow, closely packed, elongated cylindrical cells.The cells of spongy parenchyma are compactly arranged. Multicellular covering trichomes and glandular trichomes are seen.

Midrib shows the epidermal cells are polygonal in shape covered by thin cuticle. Few collenchymatous cells are present below the upper epidermis and above the lower epidermis. The ground tissue is parenchymatous. The vascular bundle is bicollateral type.

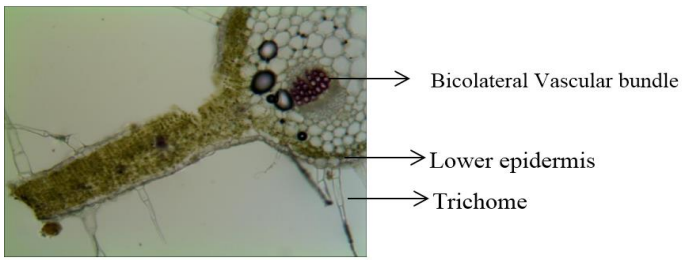


Figure 2a: T.S of *G. maderaspatana* leaf (stained)

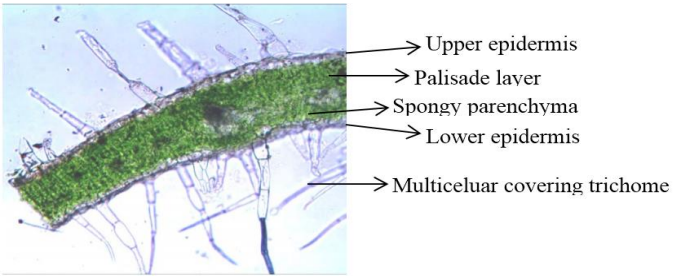


Figure 2b: T.S of *G. maderaspatana* leaf showing lamina

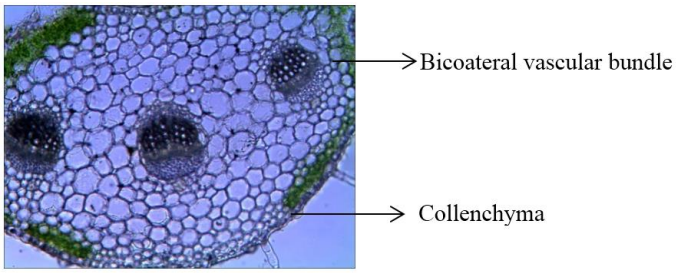


Figure 2c: T.S of *G. maderaspatana* leaf showing midrib

Stem: Transverse section of stem shows circular outline. The epidermis is single layered covered with cuticle. Epidermis consists of multicellular covering trichomes. Cortex is 6 to 8 layers thick and made up of thin walled parenchymatous cells. Some cells contain microsphenoidal calcium oxalate crystals. Endodermis is indistinct. Phloem is narrow zone consisting of 4 to 6 layers of cells. The xylem consists of small vessels and parenchyma. The pith in the centre is large and made up of thin walled parenchymatous cells.

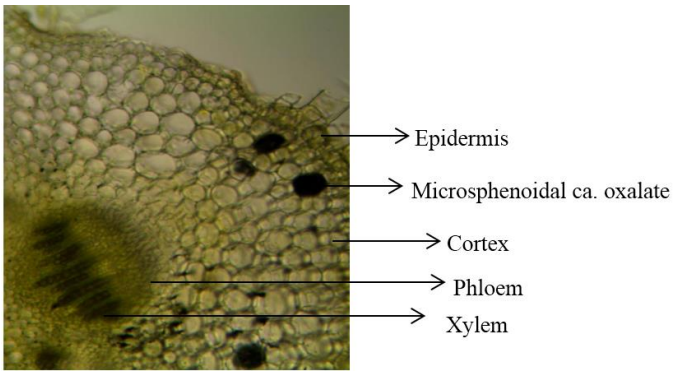


Figure 3a: T.S. of *G. maderaspatana* stem (unstained)

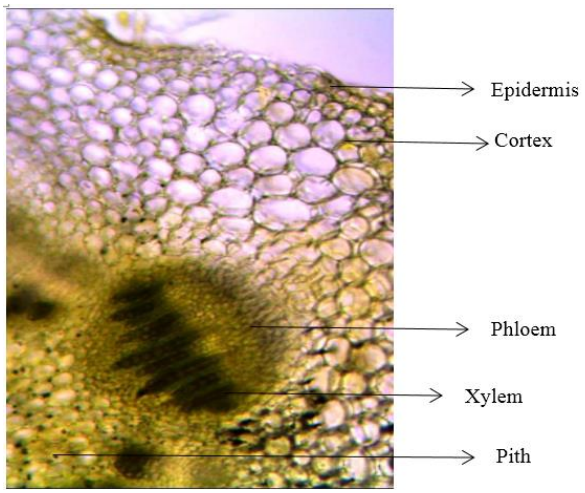


Figure 3b: T.S. of *G. maderaspatana* stem (stained)

Root: Transverse section of root shows circular outline. Cork is made up of 2-4 layers of thin walled cells. Cortex is made up of 5-7 layers of rectangular parenchymatous cells. The pericycle appers like semilunar patches of pericyclic fibres with parenchyma in between. The Phloem is 5-7 layers thick. The xylem consists of small vessels, fibres,

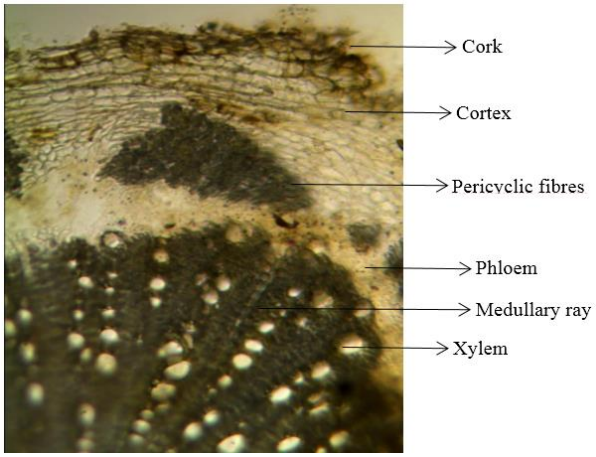


Figure 4a: T.S. of *G. maderaspatana* root (unstained)

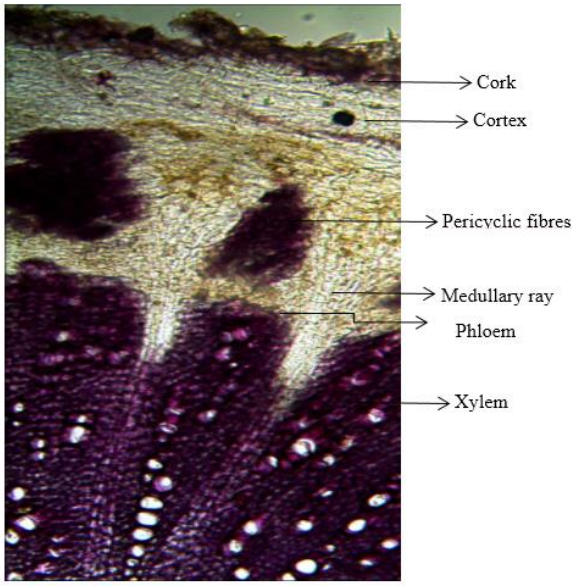
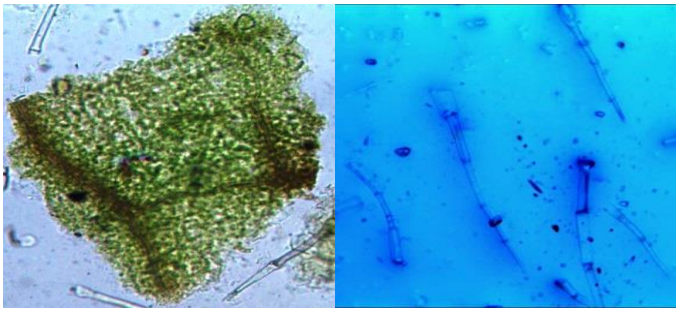


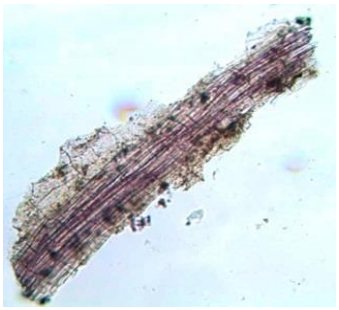
Figure 4b: T.S. of *G.maderaspatana* root (stained)

Powder characteristics

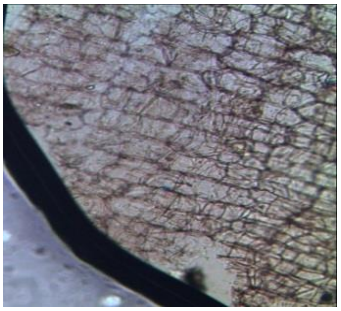
The organoleptic evaluation of powder revealed the following characteristics. The powder is light green color with characteristic odor and taste. On microscopic examination, the powder showed lamina, anisocytic and anomocytic stomata, multicellular covering trichomes, lignified fibres, Phloem, Cork and Xylem.



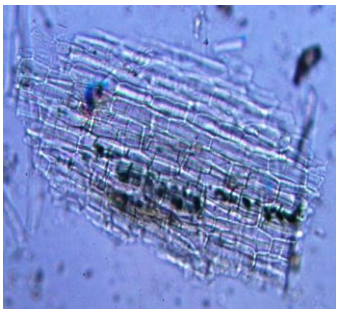
Lamina **Multicellular covering trichomes**



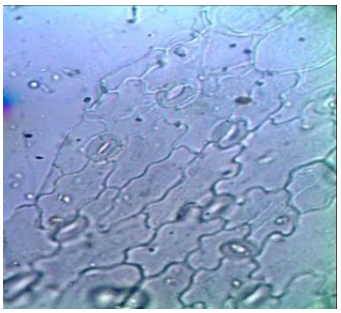
Lignified fibres



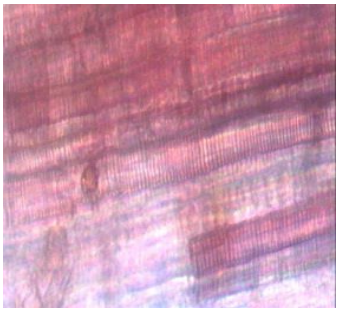
Cork



Phloem



Stomata



Xylem

(Anisocytic & Anomocytic)

Figure 5: Powder characteristics of *Grangea maderaspatana* powder

Leaf constants

The leaf constants viz., stomatal index, vein islet number, vein termination number and palisade ratio are presented in table no. 1.

Table 1: Leaf constants of *Grangea maderaspatana* leaf

Leaf constants	Values
Stomatal index	25-33
Vein islet number	2-5
Vein termination number	9-14
Palisade ratio	6.00-8.00

Physico-chemical parameters

The ash values of a drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of exhausted or adulterated drugs. The results are presented in table no. 2.

Table 2: Physico-Chemical Parameters of powder of *Grangea maderaspatana*

Sr. No.	Parameters	Values (% w/w)
1	Loss on drying	8.0%
2	Ash value	
	Total ash	11.6%
	Acid insoluble ash	1.8%
	Water soluble ash	4.5%
3	Extractive value	
	Water soluble extractive	10.0%
	Alcohol soluble extractive	10.0%

Preliminary phytochemical screening

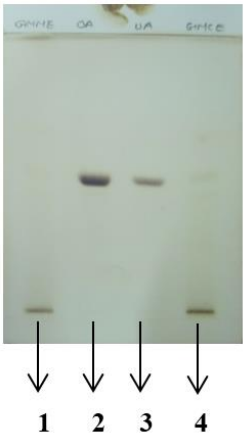
Preliminary phytochemical screening revealed presence of carbohydrates, phytosterols, saponins, terpenoids, flavonoids, tannins and phenolics (Table no. 3)

Table 3: Phytochemical screening of extracts of *Grangea maderaspatana*

Chemical constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
Carbohydrate	-	-	-	+	++
Protein	-	-	-	-	-
Phenolics & Tannins	-	-	-	+	+
Saponins	-	-	-	+	++
Flavanoids	-	-	-	+	+
Terpenes	++	++	+	+	-
Steroids	++	++	+	-	-
Alkaloids	-	-	-	-	-

(+ - Positive test, - - Negative test)

HPTLC Fingerprinting:



1. Gm. MeOH ext.
2. Std – Oleanolic acid
3. Std – Ursolic acid
4. Gm. $CHCl_3$ ext.

Figure 6a: HPTLC plate after derivatization with Anisaldehyde sulphuric acid

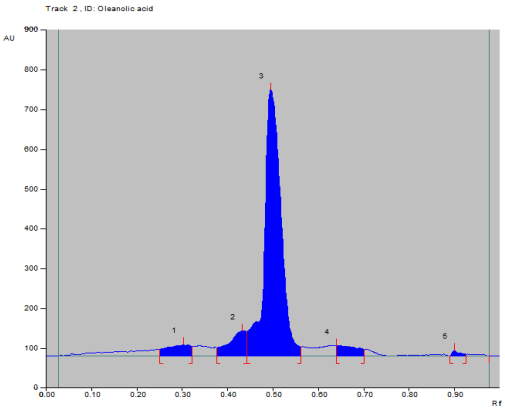


Figure 6b: Chromatogram of standard Oleanolic acid

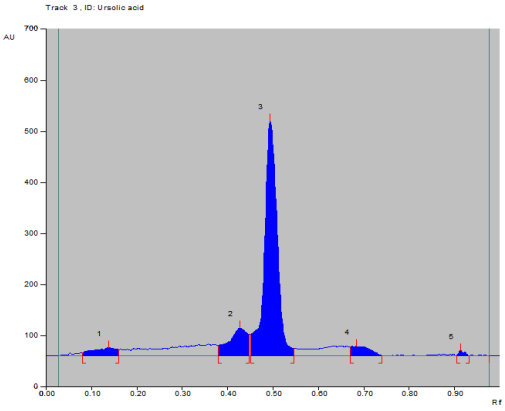


Figure 6c: Chromatogram of standard Ursolic acid

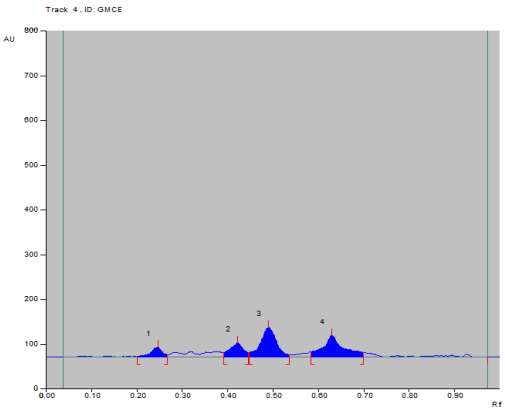


Figure 6d: Chromatogram of chloroform extract of Gm

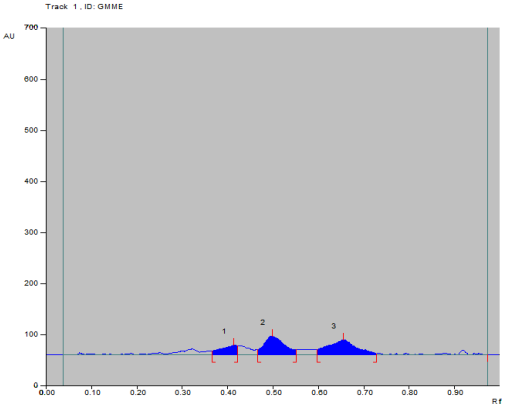
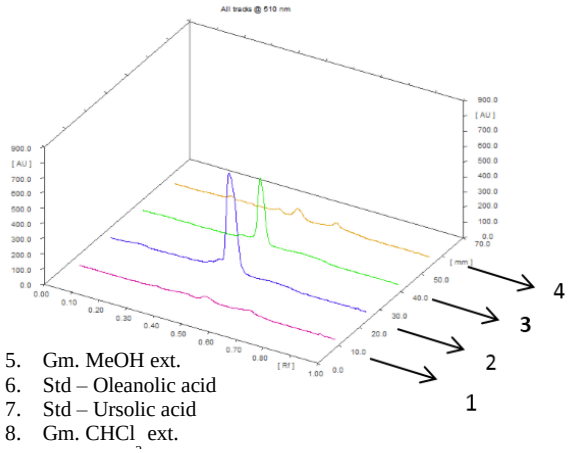


Figure 6e: Chromatogram of methanol extract of Gm



5. Gm. MeOH ext.
6. Std – Oleanolic acid
7. Std – Ursolic acid
8. Gm. CHCl$_3$ ext.

Figure 6f: HPTLC 3DChromatogram

CONCLUSSION

The microscopic character, leaf constants, phytochemical screening and physicochemical constants studied here can be used for judging the adulteration and purity of this drug. As there is no pharmacognostic anatomical work on record for this drug, the present work was taken up with a view to lay down standards which could be useful to detect the adulteration and purity of this plant

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