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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF ROOT OF *DATURA METEL* L.

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ABSTRACT: Background: *Datura metel* L. belongs to family Solanaceae is an annual or biennial shrub-like plant is native to Asia, Africa, and the Americas specifically to tropical and subtropical regions. The root of plant is used as analgesic, antipyretic, anti inflammatory, antispasmodic, wound healing, antifungal, antibacterial, sedative and hypnotic. Its significance increased due to its efficacy in case of bites of rabid dogs. Hence, Pharmacognostic evaluation of *Datura metel* L. dried Roots was carried out and reported. **Methodology:** Morphoanatomical, powder microscopic evaluation, physicochemical parameter TLC were carried out according to the API. Dried crude drug powder analysis, micro chemical evaluation, preliminary screening for phytoconstituent category were carried out as per standard protocol. **Results:** Diagnostic features of microscopic studies is characterized by presence of thick walled polygonal cork, lignified cells in cortical region, exarch xylem, lignified pitted parenchyma from xylem region and oil globules. Physicochemical parameter showed total ash (10.66%), acid insoluble ash (1.02%), water-soluble extractive (9.48%) and alcohol soluble extractive (2.46%). **Conclusions:** quality standard data for root of *Datura metel* can be used as standard for authentication and detection of adulteration.

INTRODUCTION: *Datura metel* L., also known as Devil's Trumpet or Horn of Plenty belongs to family Solanaceae. *Datura metel* (DM) is an annual or biennial shrub-like plant that grows up to 3-5 feet (90-150 cm) tall. DM is a relatively low-maintenance plant. DM is native to Asia, Africa, and the Americas specifically to tropical and subtropical regions. It has been naturalized in many parts of the world and can thrive in a variety of habitats, including waste areas, roadsides, and disturbed lands ¹.

The reported phyto-constituents in root of DM are Atropine; hyoscyamine; noratropine; norhyoscyamine; hyoscyne; apohyoscyne; norhyocine; meteliodine; tigloidine; 3 α -Tigloyloxytropine; citroside A; coniferin and paeoniflorin etc ²⁻⁴. The root of plant is used as analgesic, antipyretic, anti inflammatory, antispasmodic, wound healing, antifungal, antibacterial, sedative and hypnotic ^{1, 5-6}.

Ethnomedicinal surveys from India, Bangladesh, and other Asian countries document the external application of *D. metel* root as a paste or medicated oil for the treatment of rheumatism, arthritis, neuralgia, sciatica, inflammatory swellings, and painful joints, while root preparations have also been reported for toothache, skin disorders, asthma,

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and snake or scorpion bites in regional traditional practices (Tripathi *et al.*, 1996; Jain, 1991; Islam *et al.*, 2023). In Siddha and Unani medicine, the root is primarily used externally as an analgesic and anti-inflammatory agent for rheumatic disorders and muscular pain (Alam *et al.*, 2021).

Despite the root being so useful, the data to quality standard it is not available, hence the work of development of quality standard is carried out in this studies

MATERIAL AND METHODS:

Chemicals: Phloroglucinol, Safranin, Fast green, Dragendroff's solution and Sudan red III from Loba Chemie. Hydrochloric acid, Sulphuric acid, Sodium hydroxide, Ruthenium red, Ferric chloride and Acetic acid from s.d. fine CHEM LTD. Iodine solution from Thomas Bakers and Alcohol from CS. All chemicals used in the study were of analytical grade.

Instruments: LEICA S9D and EC4 camera (Stereo microscope), Olympus BX 43 and LC camera 30 (trinocular digital microscope) and Nikon D7200 (camera).

Plant Materials: The DM root were collected from the Kanakapura, Ramanagar district, Karnataka (12°29'01.2''N dated 11/09/2020 by Dr. V. Rama Rao. Research Officer Botany. A plant voucher specimen was kept in the botany section of the Central Ayurveda Research Institute, Bangalore. The collected dried root is kept in the Pharmacognosy section of R.A.R.I., Pune (Ref. No. IMR PCG-II /2020-22- 10c). The image of plant is given in figure 1.

Macroscopic and Organoleptic Analysis: Macroscopic and Organoleptic analysis performed for identification of plant. Plant's structure, shape, and appearance and organoleptic characters of crude drug were observed and noted⁷⁻⁹.

Microscopic Characterization: Free hand thin sections of plant tissue was examined for anatomical features after staining with Phloroglucinol and Hydrochloric acid (1:1)¹⁰⁻¹⁷.

Powder Microscopy: The sample of powder (0.5 mg) was stained with suitable reagents. The microscopical characteristics observed and noted¹⁸⁻²³.

Histochemistry: Histochemical evaluation of root section and powder were conducted to visualize and localize phytoconstituents in specific cellular components by staining with suitable reagents. The detail of staining reagent is given in **Table 1**²²⁻²⁴.

Determination of pH: The pH was determined for 5 % aqueous Solution²⁵.

Physiochemical Analysis: These experiments were conducted as per API that includes loss on drying (LOD), alcohol soluble extractive (ASE), water soluble extractive (WSE), total ash (TA), and acid-insoluble ash (AIA)²⁶⁻²⁸.

HPTLC: The analysis was conducted using HPTLC apparatus (CAMAG). Stationary phase used in study was E marck silica gel 60 F₂₅₄ plates of thickness 200 µm. The mobile phase comprised Toluene: Ethyl acetate: Formic acid (6: 4: 0.2 v/v/v). Detection of R_f band was carried out at visible, 254 and 366 nm²⁹⁻³⁰.

RESULTS: Root is cylindrical, irregular in shape. Outer surface is brown colored, rough, interrupted with rootlet's scar. Fracture is highly fissured. Color is Pale in color. It is characteristic in odor and bitter in taste. Powder is pale yellow in color. TS of root of DM showing cork that is several layered, thick walled, sub-rectangular; next to cork lie cortex which is multilayered thin walled parenchymatous polygonal cells deposited with lignified cells; vascular bundles are amphicribal, consists of xylem, phloem and fibers; xylem is composed of fibers and vessels; fibers are made up of thick walled, polygonal lignified cells; vessels are thick walled, rounded lignified cells; protoxylem present towards periphery and metaxylem lie towards centre; phloem is made up of thin walled, non- lignified cells; medullary rays are found in developing stage. Powder microscopy of root of *D. metel* L. comprises of cork in surface view showing thick walled, polygonal cells; transverse view of cork shows thin walled, quadrangular cells deposited with starch grains; surface view of cortex shows thin walled, elongated quadrangular cells; parenchymatous cells from medullary region deposited with starch grains; quadrangular, lignified pitted parenchymatous cells are present; Transverse view of fragment of vascular bundle consists of lignified xylem having

thick walled vessels and polygonal fibers; other structures present in powder are lignified vessels with pitted thickenings, elongated non-lignified fiber, brown matter and oil globules; prismatic crystal of calcium oxalate ranges from 22.22 to 50 μm in size; circular compound starch gains ranges from 2.63 to 7.89 μm in size.

Diagnostic Features: It is characterized by presence of thick walled polygonal cork, lignified cells in cortical region, exarch xylem, lignified pitted parenchyma from xylem region and oil globules.

Histochemical Test: Three separate batch samples was analyzed. The analysis result is depicted in **Table 1**.

Physicochemical Parameters: Results obtained for LOD, TA, AIA, WSE and ASE are depicted in **Table 2**.

pH was found to be 6.5283 to 6.5663.

High Performance Thin Layer Chromatography: R_f value were visualise as seen in **Fig. 3**. R_f value of band was 0.013 (grey), 0.14 (grey) and 0.27(grey), were visualised at 254 nm. R_f value of band was 0.013 (blue), 0.102 (blue) and 0.263 (blue), 0.652 (Cyan) were visualised at 366 nm. R_f value of band was 0.024 (brown), 0.06 (blue), 0.216(blue), 0.579 (blue), 0.671 (Pink) and 0.877 (blue) were visualised at visible light (Derivatised with ASR).



FIG. 1: WHOLE PLANT OF DATURA METEL L

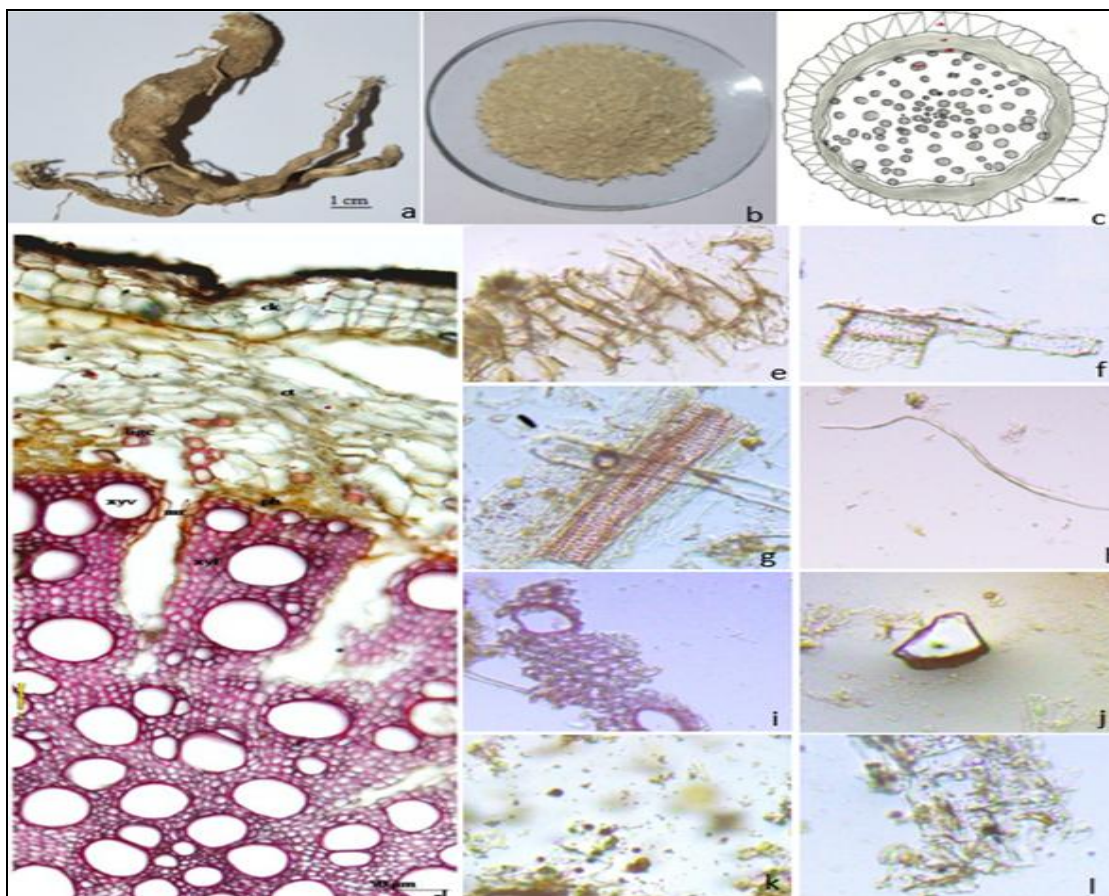


FIG. 2: A. ROOT OF D. METEL L., B. POWDER OF ROOT OF D. METEL L., C. SCHEMATIC OUTLINE DIAGRAM OF ROOT OF D. METEL L., D. TS OF ROOT OF D. METEL L. AT 10X X 10X., E TO L POWDER MICROSCOPIC CHARACTERS (E. CORK IN SURFACE VIEW, F. PITTED PARENCHYMA, G. BORDERED PITTED VESSEL, H. NON- LIGNIFIED FIBER, I. FRAGMENT OF VASCULAR BUNDLE, J. PRISMATIC CRYSTAL OF CALCIUM OXALATE, K. COMPOUND STARCH GAINS AND L: OIL GLOBULES).

TABLE 1: HISTOCHEMICAL EVALUATION OF ROOT OF DM

Test	Chemicals	Observation	Inference
Alkaloids	Dragendorff's reagent	Orange-red precipitate	+
Aleurone grains	Iodine solution	No yellowish brown to brown colour	-
Starch	Iodine solution	Blue colour	+
Mucilage	Ruthenium red	No pink colour	-
Cuticle			
Lignified cell	Phloroglucinol+HCl	Purple colour	+
Calcium oxalate crystal	HCl	dissolve	+
Calcium carbonate crystal	HCl	No effervescence	-

TABLE 2: PHYSICOCHEMICAL EVALUATION AND PH VALUE OF ROOT OF DATURA METEL L.

Parameter	Value
LOD	Not more than 7.06%
TA	Not more than 10.66%
AIA	Not more than 1.02%
ASE	Not less than 2.46%
WSE	Not less than 9.48%
pH	6.5283 to 6.5663

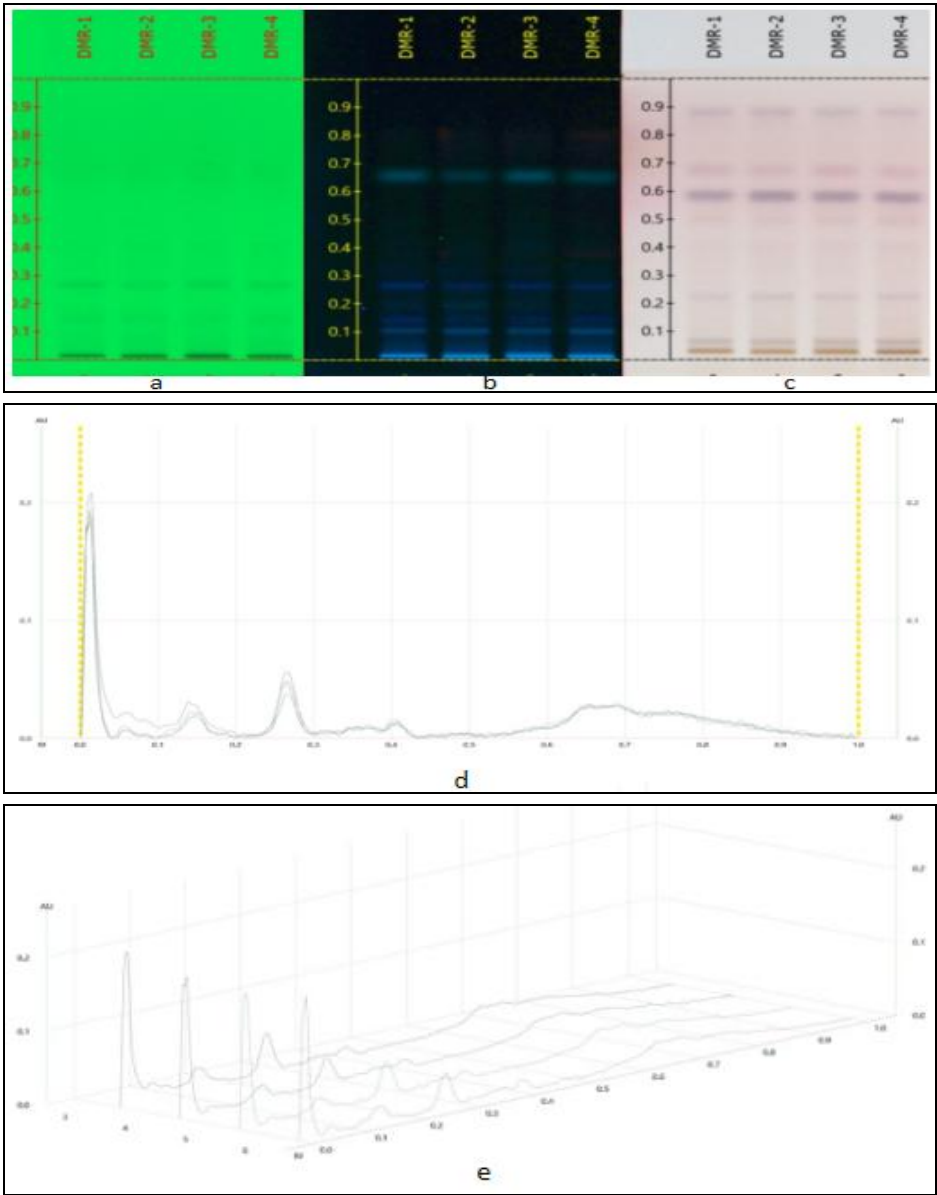


FIG. 3: HPTLC PLATES OF *D. METEL* L. (ROOT) UNDER UV 254 NM (A), UV 366 NM (B) AND VISIBLE AFTER DERIVATIZATION (C), 2D DENSITOGRAM (D) AND 3D DENSITOGRAM (E)

DISCUSSION: The present investigation establishes pharmacognostic and physicochemical quality standards for the root of DM. The root of this plant has received less attention than its leaves, flowers, and seeds despite its recognized ethnomedicinal importance. The macroscopic characters, including a cylindrical irregular root with a brown rough surface, fissured fracture, bitter taste, and characteristic odour, provide useful preliminary diagnostic features but similar gross morphological characteristics have been described for the roots of *D. stramonium* and *D. inoxia*, indicating that macroscopic characters alone are insufficient for authentication of root³¹. Microscopic features shows a multilayered cork, parenchymatous cortex, amphicribal vascular bundles, lignified fibres and vessels, exarch protoxylem, developing medullary rays, and abundant lignified tissues. These anatomical features are consistent with the general root anatomy reported for Solanaceae and particularly for *Datura* species, where secondary growth results in well-developed lignified xylem surrounded by parenchymatous cortex. Similar anatomical observations have been reported in roots of *Datura stramonium*, including multilayered cork, lignified vessels and fibres, and abundant parenchymatous tissue. However, the present study additionally demonstrates the presence of conspicuous lignified cortical cells and developing medullary rays, which may serve as supplementary diagnostic characters for *D. metel* root.

In the present powder microscopy study, the presence of polygonal cork cells, lignified pitted parenchyma, bordered pitted vessels, elongated fibres, oil globules, compound starch grains, and prismatic calcium oxalate crystals constitutes a characteristic diagnostic profile. Approximately similar diagnostic characters have been described in powdered roots of *D. stramonium* and *D. inoxia*, particularly bordered vessels, calcium oxalate crystals and starch grains³²⁻³³. Nevertheless, the simultaneous occurrence of lignified pitted parenchyma together with abundant oil globules observed in the present investigation has not been emphasized in previous pharmacognostic reports and therefore may provide additional value for identification of DM root. Histochemical analysis demonstrated positive reactions for alkaloids, lignin, starch and calcium oxalate, whereas

mucilage, aleurone grains and calcium carbonate were absent. The localization of alkaloids agrees with phytochemical studies showing that *Datura* roots are important sites of tropane alkaloid biosynthesis.

The physicochemical constants generated in this investigation provide baseline quality control parameters for *D. metel* root. The relatively low acid-insoluble ash indicates minimal siliceous contamination, while the higher water-soluble extractive than alcohol-soluble extractive suggests that hydrophilic constituents constitute a substantial proportion of the root extract. Similar observations have been reported during pharmacognostic standardization of medicinal roots, where these parameters are considered reliable indicators of purity and quality since no official pharmacopoeial standards currently exist for *D. metel* root, the values reported here provide useful preliminary reference limits for future quality assessment.

HPTLC fingerprinting demonstrated reproducible chromatographic profiles with characteristic bands under UV 254 nm, UV 366 nm and after derivatization. The generated chromatographic fingerprint can serve as an identity profile for routine authentication. Future HPTLC methods employing authentic standards of atropine, hyoscyamine and scopolamine would strengthen chemical standardization and facilitate quantitative quality control.

Compared with other species of the genus, particularly *D. stramonium*, *D. inoxia* and *D. ferox*, the overall anatomical organization of the root is highly conserved, reflecting their close taxonomic relationship. Consequently, identification based solely on gross morphology or individual microscopic characters may be unreliable. Instead, a combination of macroscopic, anatomical, powder microscopic, histochemical, physicochemical and chromatographic parameters, as presented in this study, provides a more robust approach for authenticating DM root and detecting substitution or adulteration.

CONCLUSION: The macroscopic, microscopic, powder drug analysis, histochemical analysis and physicochemical analysis revealed specific histological arrangements and physicochemical

standards and HPTLC play important role in identification of genuine sample of plants, detection of adulteration and substitution. Physicochemical parameter data helps in evaluation of quality aspects and safety aspects in preliminary form.

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CONFLICT OF INTEREST: Nil

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