Pharmacognosy Research, 2023; 15(1):101-111.
www.phcogres.com

Original Article

Pharmacognostic Evaluation and Development of Quality Control Parameters for Root of *Abelmoschus manihot* (L.) Medik

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ABSTRACT

Background: In traditional medicine, *Abelmoschus manihot* (L.) Medik, which belongs to the Malvaceae family, has been used to treat boils, sores, sprains, inflammations, tuberculosis, and leucoderma. **Objectives:** The objective of this study was to establish identification and quality control standards. **Materials and Methods:** For pharmacognostic evaluation, macroscopy, micromorphology and physicochemical constants have been used. Microchemical colour reaction tests and HPTLC studies were performed for qualitative phytochemistry. **Results:** Externally the root is yellowish-brown to yellowish-white and has a distinct odour, bitter taste, wavy shape, and smooth texture. The periderm was composed of 4-5 thin-walled cells. The secondary phloem has dilated rays and broad pyramid-shaped mucilage-packed rays. The fibres have thin walls and are narrow, and the secondary xylem is a compact circular cylinder with eight to eight radial lines of vessel chains. Prismatic calcium oxalate crystals, lignified long, narrow, thick-walled fibres with parenchyma cells, lignified cork cells, and cylindrical vessel elements were discovered in powdered samples. Physicochemically, the ash value was found above 8% which shows more than 75% solubility in acid whereas water and methanol extractive values were found to be just above and below 8% respectively. In UV-fluorescence study, very distinctive colour changes of the powdered root have been recorded. The phytochemical analysis reveals the presence of glycosides, saponins, flavonoids, tannins, and steroids. GC-MS and HPTLC are also used to characterize lipid and phenolic molecules. **Conclusion:** These findings will help in the future identification and quality control of *A. manihot* as reference standards.

Keywords: *Abelmoschus manihot*, Pharmacognosy, Physicochemistry, Phytochemistry.

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Received: 13-Sep-2022 ; **Revised:** 23-Oct-2022 ; **Accepted:** 09-Nov-2022

INTRODUCTION

Abelmoschus manihot (L.) Medik (synonym: *Hibiscus manihot*), also known as "Jangali bhendi" in India, "Huang Shu Kui Hua" in China, "Dakpul" in Korea, and "Aibika" in Indonesia, is an annual or perennial herbaceous flowering plant belongs to family Malvaceae. It is found, among other locations, in India, Sri Lanka, Nepal, China, Indonesia, Fiji, Vanuatu, New Caledonia Papua New Guinea and northern Australia among others places.[1-3]

The root of this species is used in ethnomedicine to treat boils, sores, sprains, inflammations, tuberculosis, and leucoderma.[4-7] In Nepal, the juice extracted from the roots and leaves, which has remarkable analgesic properties, is commonly and traditionally used to treat sprains.[8,9] Furthermore, numerous health foods have been commercially developed in China using the roots and other plant parts such as stems and leaves.[10]

The roots of *A. manihot* have larvicidal, anthelmintic, immune-boosting, and anti-obesity properties,[11-13] and many pharmacologically active constituents have been isolated. Pan *et al.*[14] isolated five major flavonoids from the root including rutin, hyperoside, isoquercitrin, quercetin, and myricetin. According to a review of the literature, the *A. manihot* root has not been thoroughly studied for its pharmacognostic properties. As a result, an attempt has been made to develop a preliminary pharmacognostical profile of the root that can be used as a reference standard for future research. This will help future research on the root and other plant parts, as well as other plant species. As a result, the current work was undertaken to establish specific *A. manihot* identification standards.

DOI: 10.5530/097484900263

MATERIALS AND METHODS

Plant Material

A. manihot roots were collected in Trimbakeshwar hills, Nashik district (Maharashtra). The plant was verified, and herbarium specimen number CDSAM3 (No. BSI/WC/Tech/2008/164) was deposited at the Botanical Survey of India in Pune, Maharashtra, India. The harvested plant roots were cleaned, dried, pulverized, and sieved through a 40 mesh sieve before being placed in an airtight container.

Reagents and chemicals

Analytical grade (HPLC) solvents, chemicals and reagents (Mayer's reagent, Dragendorff's reagent) were used. Research-Lab Fine Chemical Industries, Mumbai, India, and Sigma Aldrich, Germany provided all of the chemicals.

Macro and Microscopic evaluation

The root was examined macroscopically for parameters such as shape, size, colour, external features, and odour. Fresh samples of leaves, stems, and roots were fixed in FAA[15] for two days before being cut into free-hand sections with razor blades for microscopic characterization. The root sections were thoroughly boiled in chloral hydrate before being placed on slides and stained with toluidine blue.[16] The slides were then examined under a microscope and Microphotographs of the root were taken by using Jenaval and Mirax Laborec Cameras affixed to microscope.

Powder characteristics

The roots of *A. manihot* were studied under a microscope by sprinkling a little amount of powder on a microscope slide. The powdered drug was subjected to separate treatments with phloroglucinol-hydrochloric acid (1:1) solution, acetic acid, and iodine solution.[17] At various magnifications, photomicrographs were taken of characteristic structures and cell contents such as fibres, stone cells, starches, calcium oxalate crystals, and plant cells.

Physico-chemical analysis

Physico-chemical analyses were used to evaluate the levels of moisture, total ash, water-soluble ash, acid-insoluble ash, and sulphated ash. To determine the amount of water- and ethanol-soluble components, extractive values comprising ethanol- and water-soluble extractives were measured. The crude fibre content of dried powdered material of *A. manihot* roots was also determined.[18,19]

Fluorescence analysis

The standard method was used to analyse dried powdered roots for fluorescence analysis under daylight, short and long-UV light to detect fluorescent compounds following treatment with various organic/inorganic reagents.[18,20,21]

Behaviour analysis of root powder

To detect the phytoconstituents with colour changes under ordinary daylight, behaviour with chemical reagents was carried out on the crude root powder using various chemical reagents.[20]

Heavy Metal and Mineral content analysis

Instrument conditions: The Atomic Absorption Spectrometer (AAS) (Perkin Elmer-400) was used to analyze the heavy metals chromium (Cr), lead (Pb), and nickel (Ni), as well as elements such as aluminium, magnesium, zinc, copper, and iron, with argon as the carrier gas and a flow rate of 1ml/2min.

Preparation of sample solution: A sample vessel was filled with an accurately weighed 0.5 g of air-dried root powder to create ash. The sample vessel was filled with a precise 6 mL of nitric acid, and the jar was sealed with an end cap. The sample vessel was placed in the muffle furnace at 150°C as soon as a steady weight was achieved. The contents of the sample vessel were transferred to a 25 mL volumetric flask. After washing with 4.5 mL of 0.2% nitric acid, the sample vessel was transferred to the same volumetric flask. Nitric acid at a concentration of 0.2% v/v was used to adjust the volume. The solution underwent filtering and mineral and heavy metal analysis.[22,23]

Extraction and Preliminary Phytochemical analysis

The coarse powdered material of the air-dried root of *A. manihot* was defatted using petroleum ether. Using a Soxhlet extractor, the defatted material was extracted for 5 hr with chloroform, ethyl acetate, ethanol, and distilled water. The liquid extracts were then filtered to remove the solid plant components. Using a rotary evaporator, all extracts were concentrated independently.[24]

Qualitative Phytochemical analysis

The preliminary phytochemical study of different *A. manihot* root extracts were evaluated qualitatively by the corresponding chemical tests.[9,25]

GC-MS analysis of Lipids

The residue obtained after evaporation of petroleum ether extract of *A. manihot* root was subjected to saponification by 0.5 N alcoholic KOH (500mL) and refluxed for six hours, cooled and concentrated under vacuum, then mixed with 100 mL water. The unsaponifiable matter was extracted exhaustively with ether. The combined ethereal extract was washed with water until alkalinity was removed, then dried over anhydrous sodium sulphate and evaporated to dryness. The fatty acid-containing saponifiable part was dissolved in 50 mL of absolute methanol containing 5% HCl and refluxed for three hours before being cooled, diluted with water, and extracted with ether. The ethereal extract was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness before being analyzed for fatty acid methyl esters using GC/MS. On the GCMS-QP 2010 SHIMADZU instrument,

GC-MS spectra and chromatograms were recorded. V Life MDS 4.3 software is used for compound molecular modelling.[26]

HPTLC fingerprinting of methanol extract

100 mg of dried methanol extract of *A. manihot* root was accurately weighed and dissolved in 10 ml of methanol. The contents were filtered through Whatman No. 1 paper after being sonicated for 10 min. The final volume was filled to 100 ml with methanol to produce a stock solution containing 1mg/ml for HPTLC analysis, as well as the reference standards (Gallic acid) prepared in methanol at 30µg/ml. The root sample (5, 10, 15, and 20 µl) and gallic acid solution (5 and 2 µl) were applied by CAMAG LINOMAT V sample applicator equipped with a 100 µl Hamilton syringe to the HPTLC plate (Merck Silica gel 60 F_{254} 10 cm × 10 cm) as bands 8mm wide 30mm apart and 10mm from the bottom edge of the same chromatographic plate. At room temperature (30), ascending development of a distance of 80mm was performed using chloroform: ethyl acetate: formic acid (7.5:6:0.5v/v/v) as a mobile phase in a CAMAG HPTLC chamber that had been saturated for 20 min. Next, the developed plate was scanned at 292nm with a CAMAG TLC scanner using WINCAT software and a deuterium lamp after being dried at 105 C in a hot air oven until the colour of the band appeared and was visible under white light.[26,27]

RESULTS

Macro and Microscopic evaluation

The macro-morphological characterization of the root of *A. manihot* revealed that it was externally yellowish brown, with yellowish brown bark lying on yellowish-white wood. The roots have a distinct odour, a bitter taste, a wavy shape, and a smooth texture, varying in length from 3-6 cm and thickness from 0.5 to 2.5 cm (Figure 1A and B). The microscopic characterization of *A. manihot* roots revealed the general characteristics of a dicotyledonous plant. The histological examination of root sectioning revealed a continuous, thick periderm composed of four or five thin walled tabular phloem cells. The secondary phloem is a broad zone of dilated rays and broad pyramid-shaped phloem elements. The phloem zone is rich in dense masses of mucilage. Phloem rays have thin walls and are radially elongated. The phloem is made up of thin tangential or irregular fibre masses. The fibres have thin walls and are narrow. The secondary xylem is a compact circular cylinder. The secondary xylem is made up of approximately 8 to 8 radial lines of vessel chains. The vessels are angular, with thin walls, and are mostly solitary or in groups of two. The central section is narrow and composed of a cluster of primary xylem vessels. The vessels are encased in a thin-walled, wide-fibre sheath. Vascular radial chains are separated from one another by dilated rays. Ray cells have thin walls and are radially oblong. Secondary xylem and secondary phloem with phloem fibres, starch grains, and mucilage masses were found in transverse sections of *A. manihot* root. The xylem rays and phloem parenchyma are rich in starch grains (Figure 2).

Powder characteristics

Powder microscopy of the roots of *A. manihot* revealed lignified long, narrow, thick-walled fibres as well as short, wide, thin-walled fibres with parenchyma cells, lignified cork cells, and cylindrical vessel elements. Chemo-microscopy revealed the presence of lignin, starch grains, and calcium oxalate crystal clusters (Figure 3).

Physicochemical characteristics

The physicochemical parameters such as total ash, acid-insoluble ash, water-soluble ash, and sulfated ash values of *A. manihot* dry root powder were determined, and the results are shown in Table 1. Whereas. The extracts were prepared using standard methods with various solvents, and the percentages of the dry extracts were calculated in terms of air-dried crude root powder weight, as shown in Table 1.

Fluorescence characteristic

The root powder of *A. manihot* was examined in daylight, short and long-UV light to detect fluorescent compounds, and the results are shown in Table 2.

Behaviour of powdered drug

The crude root powder of *A. manihot* was tested with different chemical reagents to detect phytoconstituents with colour changes under ordinary daylight, and the results are shown in Table 3.

Heavy metal and mineral analysis

Heavy metal and mineral analysis of root powder using an atomic absorption spectrometer (AAS) revealed the presence of heavy metals such as lead 3.95 ppm, nickel 4.58 ppm, chromium 0.38 ppm, and copper 13.63 ppm, as well as minerals such as magnesium 28.05 ppm, zinc 27.88 ppm, and irons 168.03 ppm, while aluminium had not detected (Table 4).

Qualitative Phytochemical analysis

The results of qualitative phytochemical screening on all five successive dried root extracts revealed the presence of glycosides, saponins, flavonoids, steroids and tannins, etc, these are presented in Table 5.

Lipid analysis of Petroleum ether extract

The unsaponifiable portion of *A. manihot* roots petroleum ether extract was extracted exhaustively with ether. The combined ethereal extract was washed with water until alkalinity was removed, dried over anhydrous sodium sulphate, evaporated to dryness, and subjected to GC/MS analysis. The unsaponifiable portion with a reddish-brown colour yielded 0.02 g. The melting

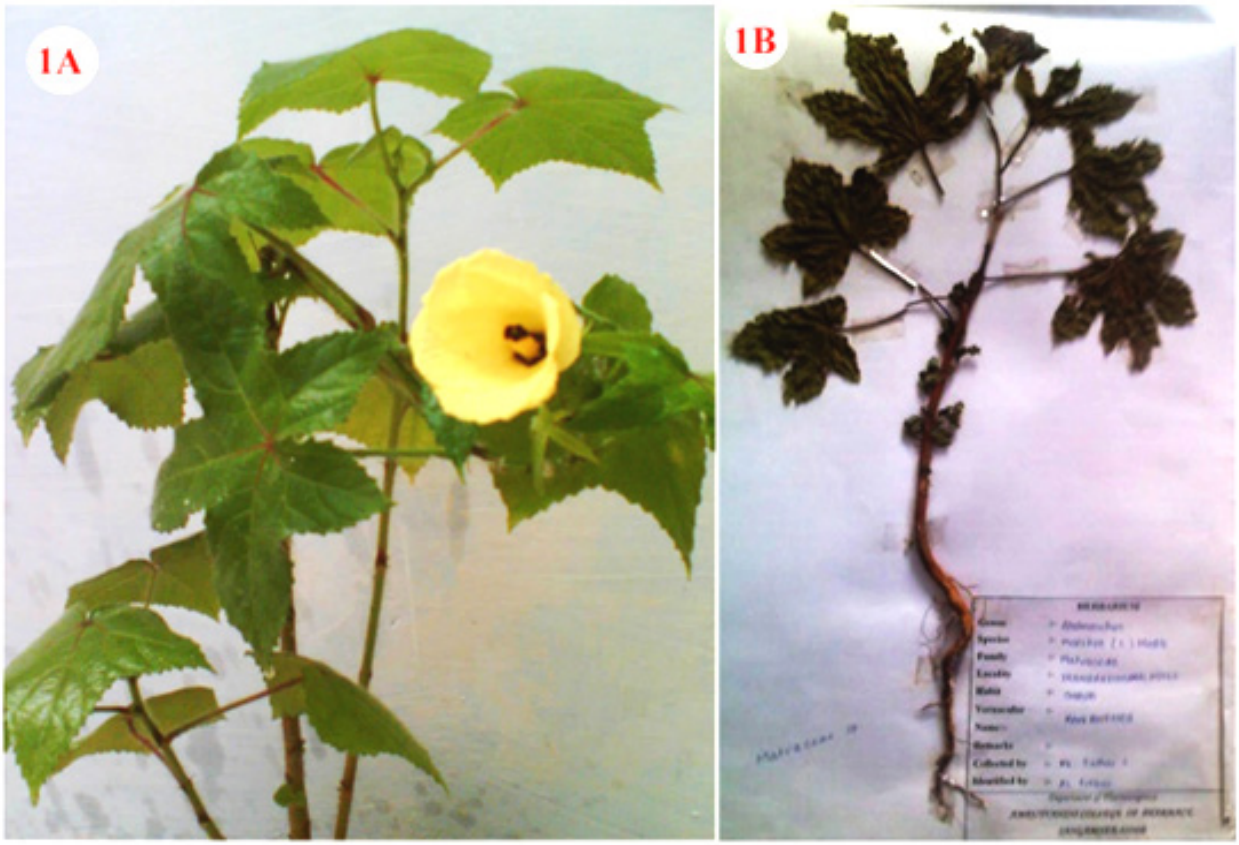

Figure 1: Morphological features of *A. manihot*- 1A: Twig; 1B: Root.

point of the unsaponifiable portion was determined to be 200-250°C. The result of GC-MS analysis of an unsaponifiable fraction of petroleum ether extract revealed that it importantly contains coumarin, propanoic acid, 4-Bromoaniline, Acetol, Benzoic acid, Benzeneacetic acid (Table 6; Figure 4).

HPTLC fingerprinting

Eleven dark bands were detected in the HPTLC fingerprint of the methanol extract of *A. manihot* roots scanned at 254 nm, with R_f values of 0.03, 0.07, 0.11, 0.19, 0.25, 0.30, 0.35, 0.49, 0.57, 0.66 and 0.77. At R_f values of 0.10, the standard compound gallic acid formed a band (Figure 5). This indicates that gallic acid is present in the plant extract. When visualised after derivatization with sulphuric acid, the root showed eleven bands at UV-366 nm, one of which, at R_f 0.11, was found to be similar in all four tracks with varying concentrations.

DISCUSSION

In this work, we used the root of *A. manihot* to standardise its pharmacognostic, physicochemical, and phytochemical characteristics. Pharmacognostic studies are the first stage in defining criteria for evaluating the authenticity and quality of every crude drug medicine. Macro and micromorphological evaluation, along with physicochemical and phytochemical analyses, have led to the identification of a great number of plant species in recent years. These techniques have uncovered cases of adulteration and substitution.[28,29] Since herbal crude drugs are so commonly used in traditional medicine, standardization is crucial for ensuring their quality, purity, and authenticity. The initial step in this process is verifying the identity of plant species. Since the morphological and anatomical investigation is among the easiest and least expensive ways to establish the correct identification of source materials.[30,31]

Here the macromicroscopic character of the root has a distinctly pungent odour, bitter flavour, wavy appearance, and smooth texture on the outside. The transverse section of the root showed the presence of 4-5 cells with a rather thin-walled periderm. Wide, pyramidal, mucilage-packed rays characterize the secondary phloem. The secondary xylem is a compact cylindrical structure with 8-8 to eight radial lines of vessel chains; its fibres have thin walls and are narrow. Before being used or sold, the majority of crude drugs are powdered. As a result, it is critical to establish standards for detecting adulteration in powdered drug samples.[29] Prismatic calcium oxalate crystals, lignified long, narrow, thick-walled fibres with parenchyma cells, lignified cork cells, and cylindrical vessel elements were discovered in powdered samples.

Quantitative physicochemical standards for *A. manihot* powdered roots include total ash, acid insoluble ash, water soluble ash, and sulphated ash values of 8.23 ± 0.16%, 1.06 ± 0.03%, 2.20 ± 0.07%

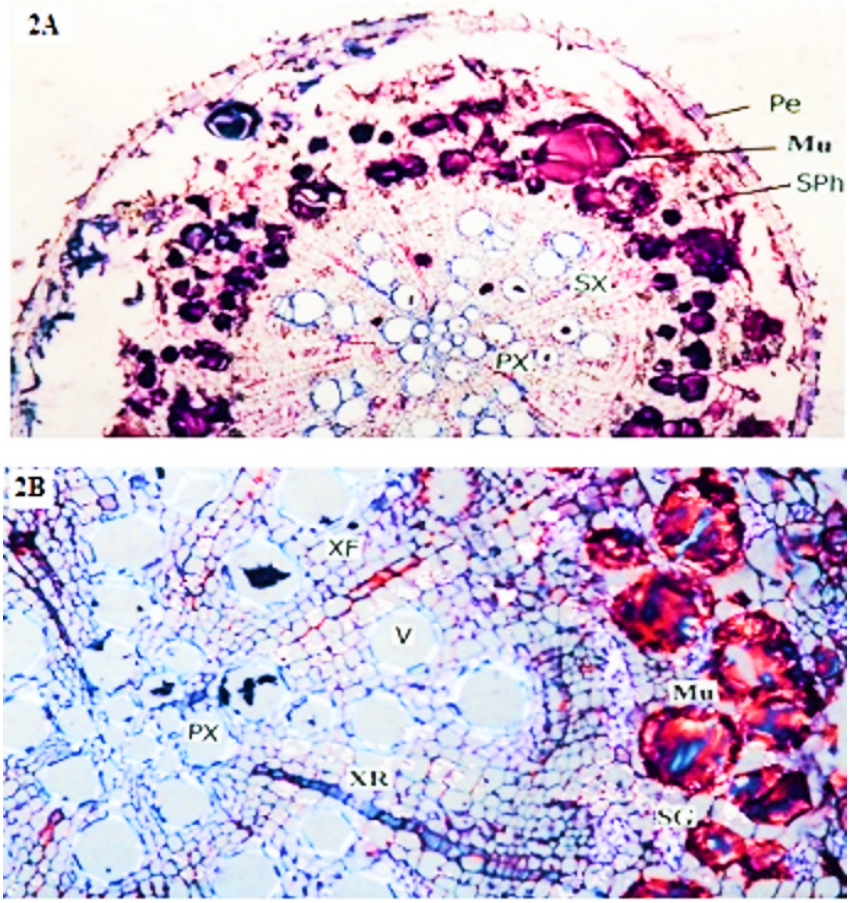

Figure 2: Transverse section of *A. manihot* roots- 2A: Periderm (Pe) and Secondary Phloem (SPh) with large dense masses of Mucilage (Mu); 2B: Primary xylem (PX) consists of vessels (V), xylem fibers (XF), starch grains (SG) in the xylem rays (XR).

and 6.41 ± 0.13% w/w, respectively. The moisture and total crude fibre content of root powder were 1.23 ± 0.09% and 44.25 ± 0.39% w/w respectively (Table 1). Crude drug identification and quality control rely on their physicochemical features. Here, it was discovered that the physicochemical values of the investigated species were significantly unique. Given that the ideal moisture level for a crude drug is less than 14%, the low moisture content (1.23 0.09) inhibits the growth of bacteria, fungi, and yeast.[32] Total ash analysis is especially useful in determining drug purity since it detects the presence or absence of foreign inorganic matter such as metallic salts or silica.[33] It was discovered that the ash value of the root is 8.23% and that it is also very distinct. The ash value of the root indicates the presence of a significant amount of inorganic minerals. Furthermore, the discrepancy between the acid insoluble ash value and the water-soluble ash value in the root of this plant emphasizes its significance in the authentication and quality control of the crude drug. The extractive value is an important part of the physicochemical analysis since it quantifies the amount of crude extract that can be recovered from different solvent extractions.[34] Furthermore, the results of the experiment show that less polar solvents, such as petroleum ether, ethyl acetate, and chloroform, have less extractive values for the root than polar solvents. Polar solvents, such as methanol, ethanol and water, extract the root more effectively than non-polar solvents. This could indicate that the root has more polar constituents than non-polar constituents (Table 1). The solvent used during an extraction process may be affected by this variation in extractive values. A lower extractive value results from the addition of exhausted material, adulteration, or incorrect processing during drying, storage, or formulation. The presence of fats, lipids, and possibly steroids in the drug is indicated by the ether soluble extractive value.[35]

Fluorescence characteristics are another quick method for resolving doubtful specimens. Plant materials can be identified and separated from their adulterants based on fluorescence characteristics when physical and chemical approaches are inadequate.[31] Many different chemical components of plants display the fluorescence phenomena. During the day, you may notice the visual fluorescence of some of the components. Even if the chemicals themselves are not fluorescent, they can often be transformed into fluorescent derivatives or decomposition products by the use of various reagents. Therefore, it is an important metric for pharmacognostical evaluation of crude medications.[36] The fluorescent colours observed for *A. manihot* powdered roots were documented under long (365 nm) and short (254 nm) wavelengths, as well as in visible light (Table

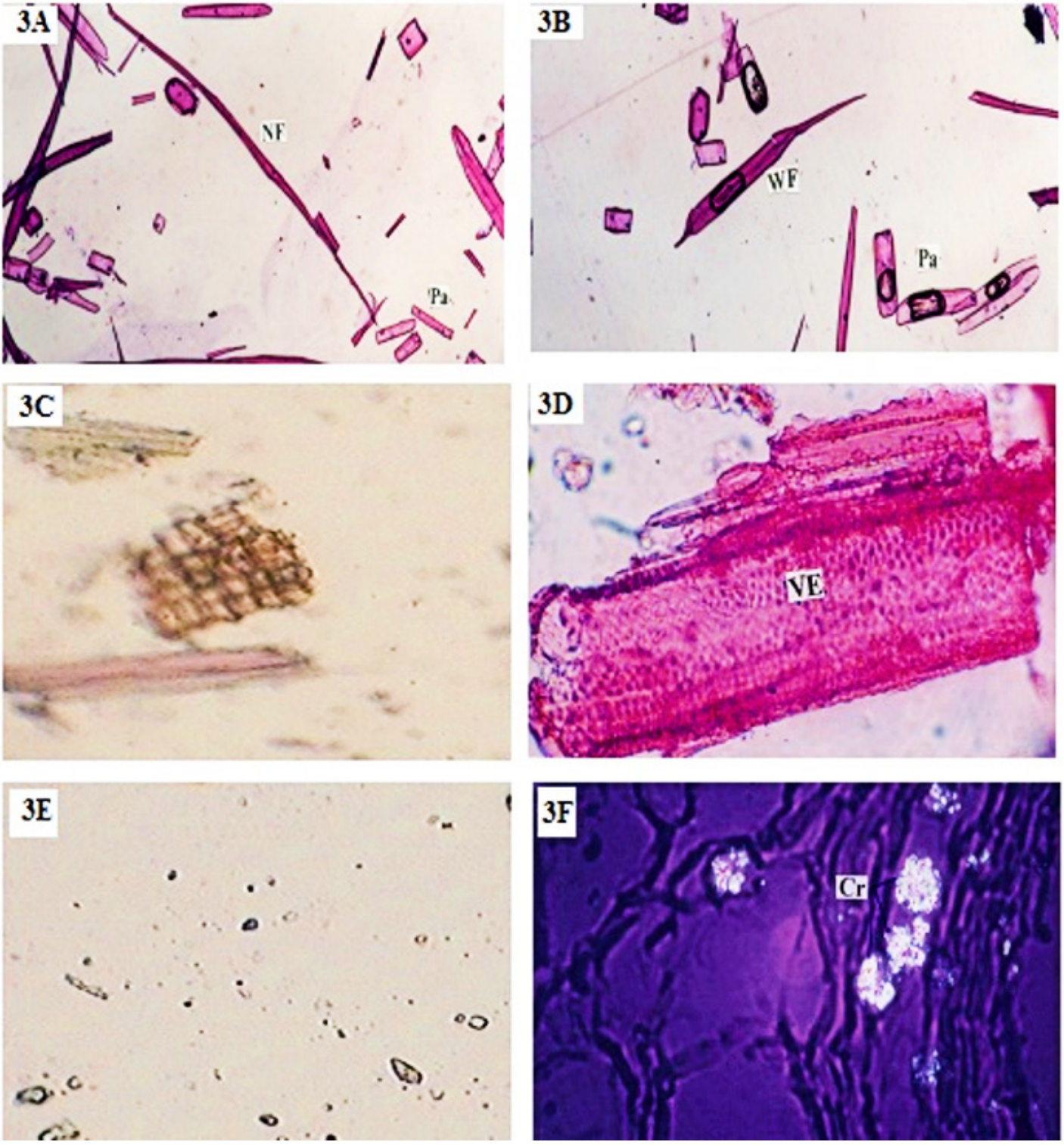

Figure 3: Powder microscopy of *A. manihot* roots- 3A: Long, thick walled narrow fibres (NF) with parenchyma cells (Pa); 3B: Short, thin walled wide fibres (WF) with parenchyma cells (Pa); 3C: Lignified cork cell; 3D: Cylindrical vessel element (VE); 3E: Simple starch grains; 3F: Cluster form of Calcium oxalate crystals (Cr).

2). Examining *A. manihot* root powder with various chemical reagents revealed a high abundance of certain phytochemicals, particularly steroids, phenolic compounds, starch, flavonoids, and proteins (Table 3). A powdered drug displayed a distinct interaction with a specific solvent or reagent. The powder floats on the surface of the majority of the study's reagents. In sulfuric acid, ferric chloride, and Magnesium-HCl, powder changes sequentially brownish-red, black, blue, and pink. In a solution of potassium hydroxide, the powder turns greenish.

Minerals such as magnesium, zinc, and iron are required components of the crude drug and play critical roles in the biosynthesis of certain secondary metabolites. Heavy metals, on

Table 1: **Physicochemical parameters of *A. manihot* root.**

Parameters	Values* (% w/w) ± SD
Moisture content	1.23 ± 0.09
Total ash	8.23 ± 0.16
Acid-insoluble ash	1.06 ± 0.03
Water-soluble ash	2.20 ± 0.07
Sulphated ash	6.41 ± 0.13
Total crude fiber content	44.25 ± 0.39
Water soluble extractive	8.3± 0.07
Ethanol soluble extractive	5.16 ± 0.01
Methanol soluble extractive	7.0± 0.13
Chloroform soluble extractive	3.3± 0.17
Ethyl acetate soluble extractive	4.2± 0.05
Petroleum ether soluble extractive	3.8± 0.02

* An average of three determinations

Table 2: **Fluorescence analysis of powdered roots of *A. manihot*.**

Reagents	Color observed in Ordinary light	Color observed under Ultraviolet light	
		Short (254 nm)	Long (366 nm)
1N NaOH in methanol	Yellowish	Blue	Black
1N NaOH in water	Yellowish	Blue	Bluish Brown
1N HCl	Yellowish brown	Blue	Green
50% HNO_3	Brown	Brown	Black
50% H_2SO_4	Yellowish brown	Brown	Black

Table 3: **Behavior analysis of powdered roots of *A. manihot*.**

Reagents	Colour/ppt	Constituents
Powder as such	Yellow	_
Conc. H_2SO_4	Brownish Red	Steroid (+)
Aqs. Ferric Chloride	Black color	Phenolic compound (+)
Aqs. Iodine Solution	Blue color	Starch (+)
Aqs. Mercuric chloride	No Brown color	Alkaloid (-)
Picric acid	No Yellow ppt	Alkaloid (-)
Magnesium- HCl	Pink color	Flavonoids (+)
Aq. Silver Nitrate Solution	No change	Protein (+)
Ammonia Solution	No change	Anthraquinone glycosides (-)
Aqs. KOH	Greenish	Anthraquinone glycosides (-)

$: (+) – Present; (-) – Absent

Table 4: Heavy metal and mineral content analysis of *A. manihot* roots.

Heavy metals and Mineral Content	Values (ppm) in root powder	Values (ppm) in root ash
Lead	3.95	3.25
Nickel	4.58	2.63
Chromium	0.38	0.16
Copper	13.63	11.39
Aluminum	N. D.	N. D.
Magnesium	28.05	25.10
Zinc	27.88	27.83
Iron	168.03	155.16

$:N. D. - Not Detected

the other hand, such as lead, may pose a health risk to consumers. The mineral levels in fresh powdered samples were determined, and the results showed that the mineral content was within WHO permissible limits, posing no risk to the consumer.[37] Initial phytochemical screening gives a general idea of the classes of active constituents in the root, which is also responsible for the therapeutic effects.[29,33] The methanol and ethyl acetate extracts contained highly polar compounds such as phenolics, tannins, and flavonoids (Table 5). Tannins and other phenolic substances with antiseptic effects may explain why this plant is used to treat a range of diseases. It has also been suggested that plant antioxidant activity may be due to phenolic compounds.[38] Flavonoids are a class of polyphenolic compounds with well-known properties

Table 5: Preliminary Phytochemical analysis of *A. manihot* roots extracts.

Chemical Constituents	Chemical tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Alkaloids	Dragendorff's test	-	-	-	-	-
	Mayer's reagent	-	-	-	-	-
Glycosides	Borntrager's test	-	-	+	+	+
	Keller-killianin test	-	+	-	-	-
Saponins	Foam test	+	+	+	+	+
Flavonoids	Shinoda Test	-	+	+	+	+
	Sodium hydroxide test	-	+	+	+	+
	Lead acetate test	-	+	+	+	+
Tannins	Ferric chloride test	-	+	+	+	+
	Phenazone test	-	+	+	+	+
Steroids	Salkowaski test	+	+	-	-	-
	Libermann-Burchard test	+	+	-	-	-

+: Present, - : Absent

Table 6: GC-MS interpretation of unsaponifiable fraction of petroleum ether extracts of *A. manihot* root.

RT (min)	Name of compound	Molecular Formula	Peak area (%)
3.134	Coumarin	$C_9H_6O_2$	11.30
9.216	Formic acid, propanoic acid, Acetic acid, Aminoguanidine)	$C_3H_6O_2$	2.06
10.642	Formic acid, propanoic acid, Acetic acid, Aminoguanidine	$C_3H_6O_2$	9.23
12.694	4-Bromoaniline	C_6H_6BrN	21.15
22.142	Acetol	$C_3H_6O_2$	2.17
23.162	Benzoic acid, Cyclopentasil oxane	$C_{16}H_{30}O_4Si_3$	3.32
24.250	Benzene acetic acid	$C_{20}H_{40}O_5Si_4$	0.10
26.329	Benzoic acid, Trimethyl ester	$C_{16}H_{30}O_4Si_3$	3.59

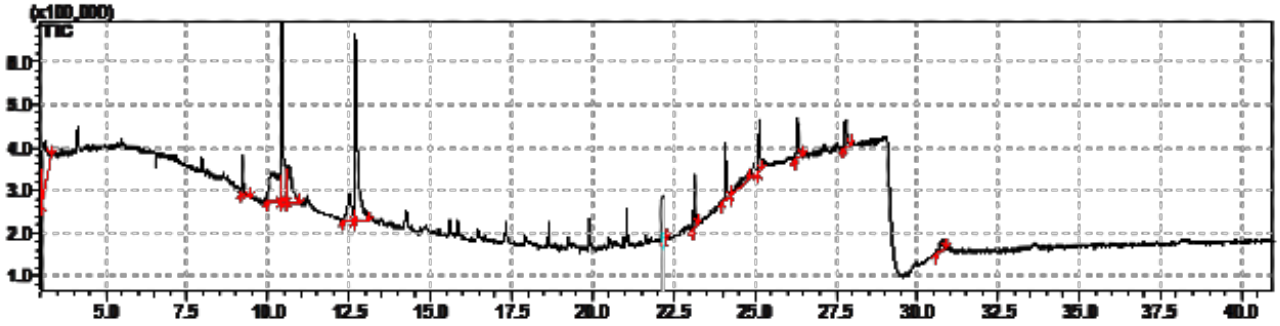

Figure 4: GC-MS Spectra unsaponifiable fraction of petroleum ether extracts of *A. manihot* root.

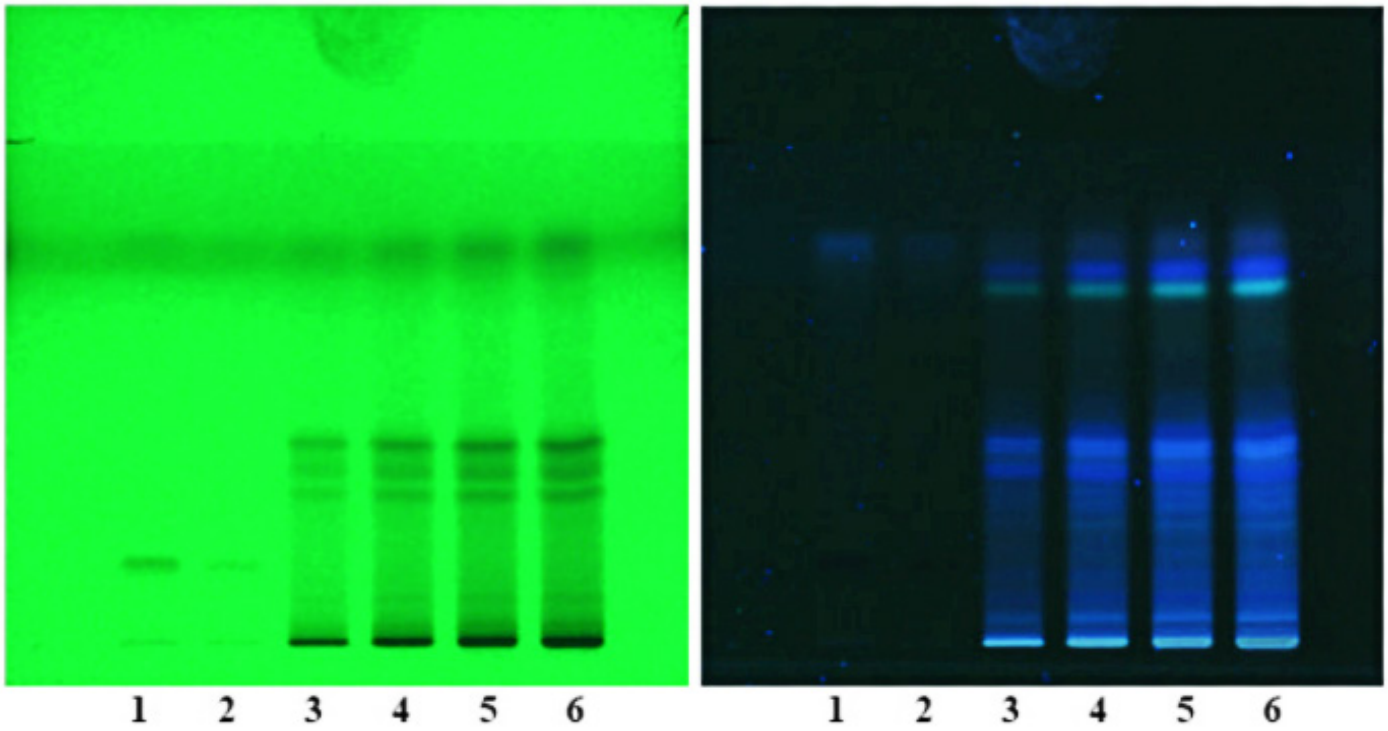

Figure 5: HPTLC fingerprinting of methanol extract of *A. manihot* root, Chromatogram spraying by 10% H_2SO_4; Chromatogram at 254 and 366 nm, Track: 1: gallic acid (5µl), 2: gallic acid (2µl), 3: root (5µl), 4: root (10µl), 5: root (15µl), 6: root (20µl).

such as free radical scavenging, hydrolytic enzyme inhibition, and anti-inflammatory activity.[39]

The GCMS analysis of a petroleum ether extract of dried roots revealed the presence of six pharmacologically active compounds. Coumarin and benzoic acid have been reported to have anti-inflammatory, anticoagulant, anticancer, antimicrobial, and antiviral activity.[40] Thin layer chromatography is still one of the quickest, cheapest, and most successful ways to obtain the unique analytical fingerprint of a plant extract, despite the availability of numerous more complex analytical techniques.[27] It is a quick and inexpensive solvent method that allows several samples to be analysed at the same time. R_f values at different wavelengths under short UV, long UV, and after post derivatization can be used as a quality fingerprint for *A. manihot* root.

CONCLUSION

This is the first study to report on the macroscopy, microscopy, powder characteristics, physicochemical parameters, heavy metals limits, phytochemical screening, Gas chromatography-mass spectrometry (GC-MS) lipid profiling, and High-performance thin-layer chromatography (HPTLC) fingerprinting of *A. manihot* root. Our findings could be used to create a standard monograph for academics, consumers, and manufacturers to use in ensuring quality assurance. Additional methods that could be investigated in future research include more sensitive chromatographic and spectroscopic chemical profiling coupled with multivariate analysis and DNA-based authentication methods.

ACKNOWLEDGEMENT

The authors express sincere thanks to Anchrom Laboratory, Mulund, Mumbai. We are also grateful to the Principal, Amrutvahini Sheti and Shikshan Vikas Sanstha's, Sangamner for providing us with the best available facility and kind support for this work.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

ABBREVIATIONS

HPTLC: High-performance thin layer chromatography; **HPLC:** High-performance liquid chromatography; **FAA:** Formalin + Acetic acid + Alcohol (ethyl alcohol); **BSI/WC:** Botanical survey of India/Western circle; **AAS:** The atomic absorption spectrometer; **GC-MS:** Gas chromatography-Mass spectrometry; **KOH:** Potassium hydroxide; **HCl:** Hydrochloric acid.

SUMMARY

This study provided the pharmacognostic data of morpho-anatomy, physicochemical and phytochemical analysis of *Abelmoschus manihot* (L.) Medik root has gained widespread acceptance in traditional medicine for the treatment of a variety of pathological manifestations. Externally, the root has a characteristic odour, bitter taste, wavy form, and smooth texture. Microscopically, the periderm had 4-5 thin-walled cells. Secondary phloem has dilated, broad, mucilage-packed rays, while phloem rays are radially elongated and have thin walls. The secondary xylem has eight to eight radial lines of vessel chains and thin, slender fibres. Powdered samples showed prismatic calcium oxalate crystals, lignified long, thin, thick-walled fibres with parenchyma cells, and lignified cork cells. Moisture content, ash values, extractive values, and crude fibre were all analyzed as physical standards. Fluorescence analysis and UV fingerprinting were also developed for the powdered extracts. In preliminary phytochemical analysis, carbohydrates, glycosides, saponins, flavonoids, tannins, steroids, and proteins were discovered. GC-MS and HPTLC were also used to characterise lipid and phenolic molecules. These findings will aid in the identification and quality control of *A. manihot* as reference standards in the future.

Authors' Contributions

DSC significantly contributed to the conception and design, data acquisition, or data analysis and interpretation. MSK participated in the article's drafting or critical revision for important intellectual content. Both authors agreed to submit to the current journal, to give final approval of the version to be published and to accept responsibility for all aspects of the work.

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