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Research Article

Pharmacognostical and phytochemical studies on roots of *Moringa oleifera* Lam.

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ABSTRACT

Context: Moringa oleifera Lam. (Moringaceae) is a well-known plant for its Abortifacient, Anticancer, Anti-inflammatory, Anti-diarrheal, Anthelmintics, and Antitumor activity. *Aims:* To study pharmacognostical, physicochemical and phytochemical investigation of roots of this plant. *Methods*: Pharmacognostical study included the macroscopic characters like size, color, surface characteristics, texture, fracture characteristics and odor of the roots. Physicochemical parameter like extractive values, loss on drying (LOD), total ash, water-soluble and acid insoluble ash of *Moringa oleifera* root powder were determined as per WHO guidelines. Preliminary phytochemical screening and qualitative chemical examination by TLC studies have been carried out for the various phytoconstituents. *Results:* Chemical evaluation and TLC studies shown presence of alkaloids, glycosides, flavonoids, steroids, saponins and tannins. The microscopic characters have shown presence of cork, xylem vessels, calcium oxalate crystals and phloem fibers. *Conclusions*: Pharmacognostical and preliminary phytochemical screening of *Moringa oleifera* roots will be useful in order to authenticate, standardize and avoid any adulteration in the raw material. The diagnostic microscopic characters and physicochemical data will be helpful in the development of a monograph.

INTRODUCTION

Moringa oleifera is the most extensively farmed Moringaceae species. It is a fast-growing tree that is used all over the world. Moringa oleifera is one of the world's most useful trees, with medical, nutritional, and other beneficial characteristics in practically every component of the tree. Plant extracts are one of the most appealing sources of new medications, with promising outcomes in the treatment of a variety of disorders such as diabetes, arthritis, and ulcers. Various components of the

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plant, including the leaves, roots, seed, bark, fruit, flowers, and immature pods, have antitumor, antipyretic, antiepileptic, anti-inflammatory, and antiulcer properties. In the ancient Indian medicinal system (Ayurveda), it is classified as a rasayana, a family of plant-derived remedies that enhance total physical and mental health and illness prevention by revitalising the body in weakened situations (Yadava et al, 2011). The Moringa oleifera plant, which belongs to the Moringaceae family, is an effective treatment for malnutrition. Moringa's leaves, pods, and seeds contain a range of important compounds, making it a nutrient-dense plant. Moringa is believed to provide seven times the vitamin C of oranges, ten times the vitamin A of carrots, 17 times the calcium of milk, nine times the protein of yoghurt, 15 times the potassium of bananas, and 25 times the iron of spinach. Moringa is a sustainable cure for malnutrition because to its ease of cultivation. Moringa is used to cure children in Senegal and Benin (Chowdhury at el, 2020).

Moringa oleifera (Moringaceae) is a highly valuable plant found in many tropical and subtropical areas. The "Drumstick" or "horseradish" tree is well-known. Moringa is a genus of 13 plants found in southwest Asia, southwest Africa, northeast Africa, and Madagascar. *Moringa oleifera, Moringa stenopetala, Moringa concanensis* and *Moringa peregrina* are the only species being studied briefly. Because the other species are unique to Madagascar and Northeast Africa, there is less investigation for naturally occurring bioactive compounds in these areas, hence they are being assessed less. *Moringa oleifera*, a plant native to India, is being investigated extensively (Gopalakrishnan at el, 2016). *Moringa oleifera* is a plant that is native to the western and sub-Himalayan regions of India, Pakistan, Asia Minor, Africa, and Arabia. It is currently found in the Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands (Ravindra at el, 2019).

MATERIALS AND METHODS

Collection of Sample

The roots of *Moringa oleifera* Lam. were collected from farm of drumstick in Amravati region in the month of February 2022. Authentication (846/21-22) of roots was done by Dr. Indrapratap S. Thakare, Department of Agriculture Botany, P. R. Pote Patil College of Agriculture, Amravati.

Macroscopic evaluation

According to WHO guidelines, the size, colour, surface characteristics, texture, fracture characteristics, and odour of the leaves were investigated (Anwar et al, 2007).

Microscopic evaluation

Free hand sectioning was used to cut root sections, which were then studied microscopically. Hydrochloric acid-phloroglucinol was used to reveal lignified elements, iodine-iodide for starch, Sudan III for lipophilic substances, Dragendorff's reagent for alkaloidal substances, ruthenium red for mucilage, ferric chloride for phenolic compounds, and silver nitrate for isothocynate glycosides were used in histochemical tests (Anwar et al, 2007).

Determination of Physico-Chemical Constituents (Torondel et al, 2014)

Determination of Loss on drying

The weight loss in percent w/w produced by the loss of water and any volatile stuff that can be driven off under certain conditions is known as loss on drying.

Procedure

2 gm of air-dried medication reduced to powder was placed in a silica crucible. The crucible was first cleaned and dried, then the weight of an empty dried crucible was calculated. A thin, equal layer of powder was applied. After that, the crucible was placed in a 105°C oven. In desiccators, the powder was dried for 4 hours and cooled to room

temperature, and the weight of the cooled crucible with powder was recorded.

- Weight of empty crucible = x g
- Weight of dried leaf powder = y g.
- Weight of Crucible + leaf powder = x + y g.
- Weight of Crucible +leaf powder after drying at 105 = z
- Loss in weight due to removal of moisture L = (x + y) - z
- % LOD = final weight/Initial weight X 100
- % LOD = L/y X 100

Determination of Total Ash value

2 gm of *Moringa oleifera* root powder were carefully weighed in a previously ignited (350^0C for 1 hour) and tarred crucible. In a muffle furnace, dried material was spread in an even layer in the crucible and the material lit by progressively increasing the heat to 550^0C for 5 hours until it was white, signifying the absence of carbon. Weighed after cooling in a desiccator. The total ash content of the material was measured in milligram per gram of air-dried material**.**

Determination of Acid- insoluble Ash

- Total ash obtained in the previous step boiled for 5 minutes in a 100 ml beaker with 25 ml dil. HCl.
- The insoluble matter was collected and washed with hot water.
- Ignited to a fixed weight.
- Insoluble in acid 100/Y = Ash (y)
- Where: Y is the weight of insoluble matter in grams,

and y is the weight of ash in gm.

Determination of Water-soluble Ash

- Total ash obtained in the previous step boiled for 5 minutes in a 100 ml beaker with 25 ml water.
- The insoluble matter was collected and washed with hot water. Ignited to a fixed weight.
- The percentage of water-soluble ash was calculated using air dried drug.

Determination of moisture content

This step was completed by inserting approximately 1.0 g of *Moringa oleifera* root powder in a correctly weighed moisture disc (Electronic measurement scale- mettler Toledo). It was dried in an oven at 105°C for 3 hours, chilled in a desiccator for 30 minutes, and then weighed immediately to estimate loss on drying. The weight loss was computed as the content of air-dried material in percent.

Preparation of extracts

The plant material was collected, dried in the shade, and powdered into a coarse powder. Soxhlet extraction was used to prepare a complete extract as well as sequential solvent extracts from the powder obtained. The plant material was collected, dried in the shade, and powdered into a coarse powder. Soxhlet extraction was used to prepare a complete extract as well as sequential solvent extracts from the powder obtained (Sholapur & Patil, 2013).

Preparation of successive extracts (Mukherjee, 2008)

The solvents were extracted one after the other in decreasing sequence of polarity, as shown below: Petroleum ether>Chloroform>Ethyl acetate>Methanol> Ethanol>Water.

One hundred grams of *moringa oleifera* root powder was packed loosely in a thimble and inserted in the Soxhlet extractor's body. The round-bottom flask was then filled with 500 ml of ethanol (solvent). The Soxhlet extractor, round-bottom flask, and condenser were then attached to the apparatus using clamps and a stand. The condenser was linked to the rubber tube connected to the tap water for continuous water flow. The dismantle was used to heat the solvent, which then began to evaporate as it passed through the apparatus to the condenser. The condensate was then dripped into the plant extract reservoir. When

the solvent level reached the syphon, it spilled back into the flask, restarting the cycle**.** The procedure was set to run for 6 hours. Finally, the extract was collected in the flask with the round bottom. The ethanol was evaporated using an IKA rotary evaporator at 40°C once the process was completed, leaving a tiny yield of extracted plant material (approximately 2–3 ml) in the glass-bottom flask. The extract was maintained in a porcelain bowl until all of the ethanol had evaporated. Using a computerized weighing balance, the quantity of extractable matter was estimated in mg/g of air-dried material. The extract was stored in the refrigerator till further use.

Preliminary Phytochemical Screening (Goswami & Singhai, 2015)

The samples were tested for several phytochemicals using standard procedures as quoted by Dr. K.R. Khandelwal (29th Edition 2018) to identify the phytochemical.

Apparatus: Test tubes, Test tube holder, Test tube stand

Test for Carbohydrates

Molisch's test (General test): To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol shake and add conc. H_2SO4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Test for Alkaloids

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute Shake well and filter. With filtrate, perform following tests: Wagner's test: 2-3 ml filtrate with few drops Wagner's reagent gives reddish brown ppt.

Test for Cardiac Glycosides

Keller-Killiani test:

To 2 ml extract, add glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.

Test for Flavonoids

To small quantity of residue, add lead acetate solution. Yellow coloured precipitate formed.

Test for Tannin

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents:

Dilute iodine solution: transient red colour.

Test for Steroids

Salkowski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml conc. H_2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for Fat and Oil

Press powder of crude drugs between two filter paper. Filter paper gets permanently stained due to oil.

Test for Saponin

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent stable foam observed.

Test for Protein

Biuret test: To 3 ml test solution add 4% NaOH and few drops of 1 % $CuSO_4$ solution. Violet or pink colour appears.

Qualitative analysis for different chemical constituents

Thin Layer Chromatography (TLC) (Kalluri, 2018)

Adaptability, rapidity, and sensitivity are just a few of the benefits of TLC over paper chromatography. TLC is an adsorption chromatography technique that uses the interaction of tiny layers of adsorbent on a plate to separate items. The method is usually used to separate molecules of low molecular weight. Thin Layer Chromatography was used to calculate the Rf value, which was done in a Twin through chamber with a silica gel 60 F254 pre coated aluminium plate with a 0.2 mm thickness and an ethyl acetate: methanol (1:1) developing solvent system.

TLC stands for thin layer chromatography and is a chromatographic technique for separating

mixtures. M. Tswett discovered chromatography in 1906. Thin layer chromatography is done on a glass, plastic, or aluminium foil sheet that has been covered with a thin layer of adsorbent material, commonly silica gel, aluminium oxide, or cellulose (blotter paper). The stationary phase refers to the adsorbent layer. A solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action after the sample has been put to the plate. Separation is achieved because different constituents ascend the TLC plate at different rates. The mobile phase and detecting reagents of various classes of compound are shown in Table 1.

RESULTS

Macroscopic Evaluation:

The morphological research demonstrated that the shape of *Moringa Oleifera* roots occurs in its whole. The root has a more or less cylindrical shape with a slight taper. Unpeeled roots are yellowish brown to dark brown in colour, while peeled roots are pale yellow with a rough surface as shown in Table 2.

Microscopic Evaluation:

Young Root

Young root shows circular in outline having elliptical epidermis followed by a zone of cortex. The cortex region divided into two zones, the outer zone composed of small parenchymatous cells often interrupted by patches of sclerenchyma; and inner zone having thin-walled large parenchymatous cells. Outer cortical cells often filled with prismatic crystals of varying shape and size. The inner few parenchymatous cells often similar to endo dermal cells in shape and size. The endodermis composed of barrel shaped cells showing casparian bands along with a few passage cells. Pericycle clearly not distinguishable below endodermis. Radial vascular bundle present having tetrarch xylem. The pith was absent in the root.

Mature Root

Transection of the root revealed circular outline. Outermost layer cork, broad approximately 7-12 layered; Cork cells arranged radially and rectangular in shape. The growth of the cork cells were not continuous around the margin but, interrupted at regular intervals. The cortical region broad with thin, isodiametric parenchymatous cells without inter-cellular space; interrupted by zone of sclerenchymatous fibres confined to outer region. Some dark pigmented myrosin cells and few crystals present in this region. These crystals were varying in shape and size, prismatic type ranging from 30-40 x 20-30 µm and stellate type ranging from 38-48 x 42-50 µm; a few oil globules and resinous matter were also scattered in the cortical region. Xylem vessels exhibited irregular distribution. Some of the larger vessel elements contained tyloses in them. Pith was also absent in the mature roots.

Physicochemical Determinations

Physicochemical parameter like Extractive values, LOD (Loss on drying), Ash value, acid insoluble ash, water soluble, moisture content of *Moringa oleifera* root powder were determined as per WHO guideline.

The results are shown in Table 3.

Extraction of Plant Material

Successive solvent extraction values in various organic solvents were observed as shown in Table 4.

Preliminary Phytochemical Screening

Chemical tests on all of the extracts obtained after successive extraction revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, tannins and steroids. The results are summarizing in Table 5 & 6.

Qualitative evaluation by Thin Layer Chromatography

Thin layer chromatography on silica was performed on approximately 20 g of the extract. The systematic order of solvent selection

demonstrates the effect of polarity on the extraction and the extracted phytochemicals.

During the thin layer chromatography procedure, the fractions with different Rf values were separated. Table 7 shows results of observation of phytochemical analysis of *Moringa oleifera* by observing the spots on TLC plates.

DISCUSSION

Morphology of *Moringa oleifera* root bark has a whitish grey colour, and is surrounded by thick cork, roots have the taste of horseradish and that are usually grows as high as 9 m. Cortex region have divided into outer and inner region, that outer zone consist of small parenchymatous cells and inner zone consist of thin-walled large parenchymatous cells. Loss on drying, Total ash, Acid insoluble ash, Water soluble ash and extractive values are all included in the physicochemical parameters. The phytochemical evaluation of various phytoconstituents in a successive extract of the roots of *Moringa oleifera* which includes alkaloid, carbohydrate, cardiac glycoside, tannin, saponin and fat and oils.

Pharmacognostical and preliminary phytochemical screening of *Moringa oleifera* roots will be useful in order to authenticate standardize and avoid any adulteration in the raw material. The diagnostic microscopic characters and physicochemical data will be helpful in the development of a monograph. According to the literature, the plant *Moringa oleifera* is a significant source of many pharmacologically and medicinally important phytoconstituents. Additionally, *Moringa Oleifera* root extracts can be subjected to pharmacological screening due to the presence of several phytochemicals that may have therapeutic activity.

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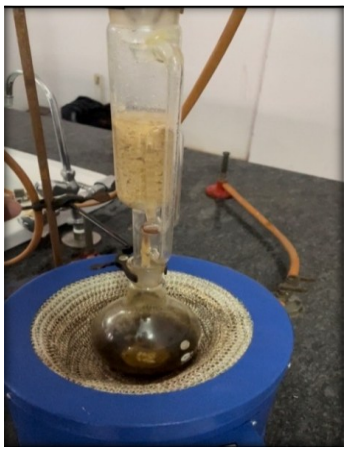

Fig. 1: Soxhlet Extraction

Fig. 2: Extractive Samples

Table 1: TLC Profile of *Moringa oleifera* root extracts (Sankeshwari et al, 2018)

Sr. No.	Groups	Mobile Phase	Detection
01	Carbohydrate	Ethyl acetate:Propanol:Water (4:1:2)	Stal's indicator
02	Alkaloids	Methnol:Water:Ammonia (70:20:10)	Dragendroff reagent
03	Cardiac glycosides	Chloroform:Acetone (7:3)	UV 365nm
04	Flavonoids	Ethyl acetate:Formic acid:Glacial acetic acid:Water (100:11:11:26)	Aluminium chloride reagent
05	Tannins	Chloroform:Methanol:Water (65:35:10)	UV 365nm
06	Steroids	Cyclohexane:Diethyl ether:Ethyl acetate (4:3:2:5)	Anisaldehyde Sulphuric acid
07	Fat and Oil	Toluene:Ethyl acetate (93:7)	Vanillin Sulphuric acid reagent
08	Saponin	Chloroform:Glacial acetic acid:Methanol:Water (64:32:12:8)	Vanillin Sulphuric acid reagent
09	Protein	Butanol:Acetic acid:Water (4:1:1)	UV 365nm

Table 2: Organoleptic features of *Moringa Oleifera* roots

Sr. No.	Features	Observations
01	Shape	Cylindrical
02	Width	1-5 cm
03	Length	20-50 cm
04	Colour	Unpeeled-Yellowish Brown To Dark Brown Peeled- Pale Yellow
05	Odour	Faint And Characteristic
06	Taste	Characteristic, Free From Bitterness.

Table 3: Physicochemical parameters

Sr. No.	Physicochemical parameters	Physicochemical parameters
1	Loss on drying	0.9078%
2	Total ash value	7.5%
3	Acid insoluble ash	2.5%
4	Water soluble ash	5.1%
5	Moisture content	2.1%

Table 4: Percentage yield of successive extraction

S.N.	Solvent	Percentage
1	Diethyl Ether	4.77%
2	Benzene	3.25%
3	Chloroform	3.43%
4	Ethanol	20.2%
5	Water	11.78%

Table 5: Observation of Phytochemical tests of *Moringa oleifera* root extract

Chemical constituents	Diethyl ether extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract
Carbohydrates	-	+	+	+	+
Alkaloids	-	+	+	+	+
Glycosides	+	+	+	+	+
Flavonoids	-	-	-	-	-
Tannins	+	+	+	+	+
Steroids	+	-	-	-	-
Fat and oil	+	-	-	-	-
Saponin	+	+	+	+	+
Proteins	-	-	-	-	-

'+' = present and significant; '-' = absent.

Table 6: Results of Preliminary Phytochemical screening of successive extracts

Sr. No.	Extracts	Results
1	Diethyl ether	Glycosides, Tannins, Steroids, Fat and oil, Saponin
2	Benzene	Carbohydrate, Alkaloids, Glycosides, Tannins, Saponin
3	Chloroform	Carbohydrate, Alkaloids, Glycosides, Tannins, Saponin
4	Ethanol	Carbohydrate, Alkaloids, Glycosides, Tannins, Saponin
5	Water	Carbohydrate, Alkaloids, Glycosides, Tannins, Saponin

Table 7: Rf value of isolated compounds of *Moringa oleifera* extract

Chemical constituents	Retention factors of different extract				
	Diethyl ether extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract
Carbohydrates	Absent	0.80	0.55	0.40	0.30
Alkaloids	Absent	0.70	0.60	0.45	0.35
Glycosides	0.65	0.50	0.45	0.50	0.60
Flavonoids	Absent	Absent	Absent	Absent	Absent
Tannins	0.80	0.58	0.45	0.40	0.75
Steroids	0.80	Absent	Absent	Absent	Absent
Fat and oil	0.80	Absent	Absent	Absent	Absent
Saponin	0.20	0.25	0.30	0.30	0.40
Proteins	Absent	Absent	Absent	Absent	Absent