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Pharmacognostic and phytochemical investigations on the bark of *Moringa oleifera* Lam.

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Moringa oleifera Lam. belonging to the Moringaceae family is a highly valued plant, distributed in many countries of the tropics and subtropics. It's some of the common names include Horseradish tree, Drumstick tree, Benzolive tree, Shajna, Nugge mara and Sigru. The bark is widely used as emmenagouge, rubefacient, anticancerous, antitubercular, antifungal, cardiac and circulatory stimulant. It is necessary to ascertain the authenticity of this crude drug when it is used for therapeutic purpose. Hence, the present study was under taken for systematic pharmacognostical evaluation of the bark of the plant with respect to macroscopy, microscopy and physico-chemical parameters. The TLC profile was developed for petroleum ether and ethanolic extract of the bark. Preliminary phytochemical investigation indicated the presence of carbohydrates, triterpenoids, isothiocynate glycosides, tannins and steroids. These established parameters could be used in identification and authentication of the crude drug.

Keywords: *Moringa oleifera*, Horseradish tree, Drumstick tree, Bark, Macroscopy, Microscopy, Fluorescence analysis, Phytochemical.

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Introduction

Moringa oleifera Lam. syn. *M. ptreygosperma* Gaertn. (Family-Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics[1]. The tree ranges in height from 5-10 m and is found wild and cultivated throughout the plains, especially in hedges and in house yards, thrives best under the tropical insular climate and is plentiful near the sandy beds of rivers and streams[2]. It has drooping branches and stems brittle, with corky bark; leaves feathery, pale green, compound, tripinnate; flowers fragrant, white or creamy-white; pods pendulous, brown, triangular, splitting lengthwise into 3 parts when dry and tapering at both ends; seeds dark brown, with 3 papery wings; main root thick[3]. It is known as the Horseradish tree, Drumstick tree, Benzolive tree, Kelor, Marango, Mlonge, Moonga, Mulangay, Nebeday and Ben oil tree[4] in various languages. In India it is locally known as ***Shajna*** (Hindi), Nugge mara (Kannada), Sigru (Sanskrit)[5]. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods are used for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

Traditionally the plant is widely used, such as the bark as emmenagouge and antifungal, the seeds as aphrodisiac. Leaves and root are anthelmintic, antitubercular, antispasmodic, abortifacient, antilithic, antifertility, anti-inflammatory, antitumors and as cardiotonic[1,6,7] and the flower possesses anti-inflammatory, antitumour and anti-hypercholesterolaemic properties[8].

The plant is a rich source of various phytochemicals, viz. the bark is reported to contain two alkaloids namely moringine and moringinine[9], phytosterols like β-sitosterol and β–sitostenone[10], glucosinolates like 4-(alpha-l-rhamnopyranosyloxy)-benzylglucosinolate[11]. The seeds contain glucosinolates like 4-(alpha-l- rhamnopyranosyloxy)-benzylglucosinolate[11], O-ethyl-4-a-L-rhamnosyloxy) benzylcarbamate[12]. The leaves contain glucosinolates like 4-(alpha-l-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of this glucosinolate[11], nitrile glycosides niaziridin and niazirin[13], isothiocyanate like 4-[(4'-O-acetyl-a-i-rhamnosyloxy)benzyl][14], acetylated glycosides bearing groups like thiocarbamate, carbamate or nitrile[15], thiocarbamate glycosides niaziminin A and B[10], phenols like quercetin-3-O-

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glucoside and quercetin-3-O-(6"-malonyl-glucoside), kaempferol-3-O-glucoside, kaempferol-3-O-(6"-malonyl-glucoside), 3-caffeoylquinic acid and 5-caffeoylquinic acid[11]. Since there are no reports of systematic pharmacognostical and phytochemical studies on the bark, the present work was planned to study the detailed macroscopical, microscopical, physicochemical and chromatographic characteristics of the bark of *M. oleifera* Lam. which would serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants.

Materials and Methods

Plant material

The fresh bark of the plant was collected in the month of November and December from the local areas of Hubli–Dharwad, Karnataka, India. The bark was authenticated by botanist Dr. Ganesh R. Hegde Professor P. G. Department of Botany, Karnataka University, Dharwad and a voucher specimen bearing no. DOUN09017 is maintained in the herbarium of Department of Pharmacognosy of KLE'S College of Pharmacy, Hubli.

Macroscopy

Macroscopic or organoleptic characters like appearance, taste, colour and odour were evaluated.

Microscopy

Bark sections were cut by free hand sectioning and numerous sections were examined microscopically. Histochemical tests were carried out using hydrochloric acid-phloroglucinol 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[4]. Photomicrographs of the microscopical sections were captured with the help of Olympus trinocular research microscope CX-21 with Digieye camera using Caliper plus version 4.2 software.

Powder characteristics

The dried bark was subjected to size reduction to get coarse powder and then the uniform powder of 40 mesh sizes. The sieved powder was mounted in and seen under microscope at 15 × 10 X magnification of the trinocular research microscope. Preliminary examination, fluorescence analysis and behavior of powder with different chemical reagents and microscopical examinations were carried out[16,17].

Physico-chemical parameters

Percentage of total ash, acid-insoluble ash, water soluble ash, sulphated ash and loss on drying were calculated[18]. Various extracts were prepared for the study of extractive values of the bark[19].

Fluorescence analysis

The powdered sample was treated with different chemical reagents to observe various colour reactions which may help to confirm the purity of the drug[20].

Preliminary phytochemical studies

For preliminary phytochemical analysis, powdered bark was extracted separately with petroleum ether (60-80°C) and ethanol 80% v/v by the method of maceration. The extracts were concentrated under vacuum in rota evaporator at 40°C dried and weighed. Each extract was tested for the presence of phytoconstituents, viz., carbohydrates, proteins, amino acids, steroids, terpenoids, glycosides, tannins, alkaloids and flavonoids[20,21]. The TLC of the extracts was performed using silica gel G60 as adsorbent. The petroleum ether extract of the bark was chromatographed using benzene and hexane in the ratio of 20:30 as mobile and 5% ethanolic phosphomolybdic acid detecting reagent, followed by heating at 115°C for 5 minutes. The ethanolic extract of the bark was chromatographed using chloroform and methanol in the ratio of 85:15 as mobile phase and 5% ethanolic phosphomolybdic acid detecting reagent, followed by heating at 115°C for 5 minutes.

Results and Discussion

Macroscopy

The bark is grey or dark green and rough externally, internally light brown or cream coloured and smooth the pieces of 5-8 cm wide and 10-20 cm in length were selected for present study. Taste none, odour characteristic, fracture-splintery and deeply fissured externally. The bark is corky and light in weight.

Microscopy

The transverse section of the bark showed cork, cork cambium and secondary cortex (Plate 1). The outer most 25-30 layered cork cells arranged in radial rows contain rectangular suberized walled cells arranged in layers. The cork layers are followed by 5-10 layered cork cambium containing multilayered thin walled rectangular cells. Secondary cortex is composed of thin walled parenchymatous cells containing calcium oxalate crystals, starch grains and

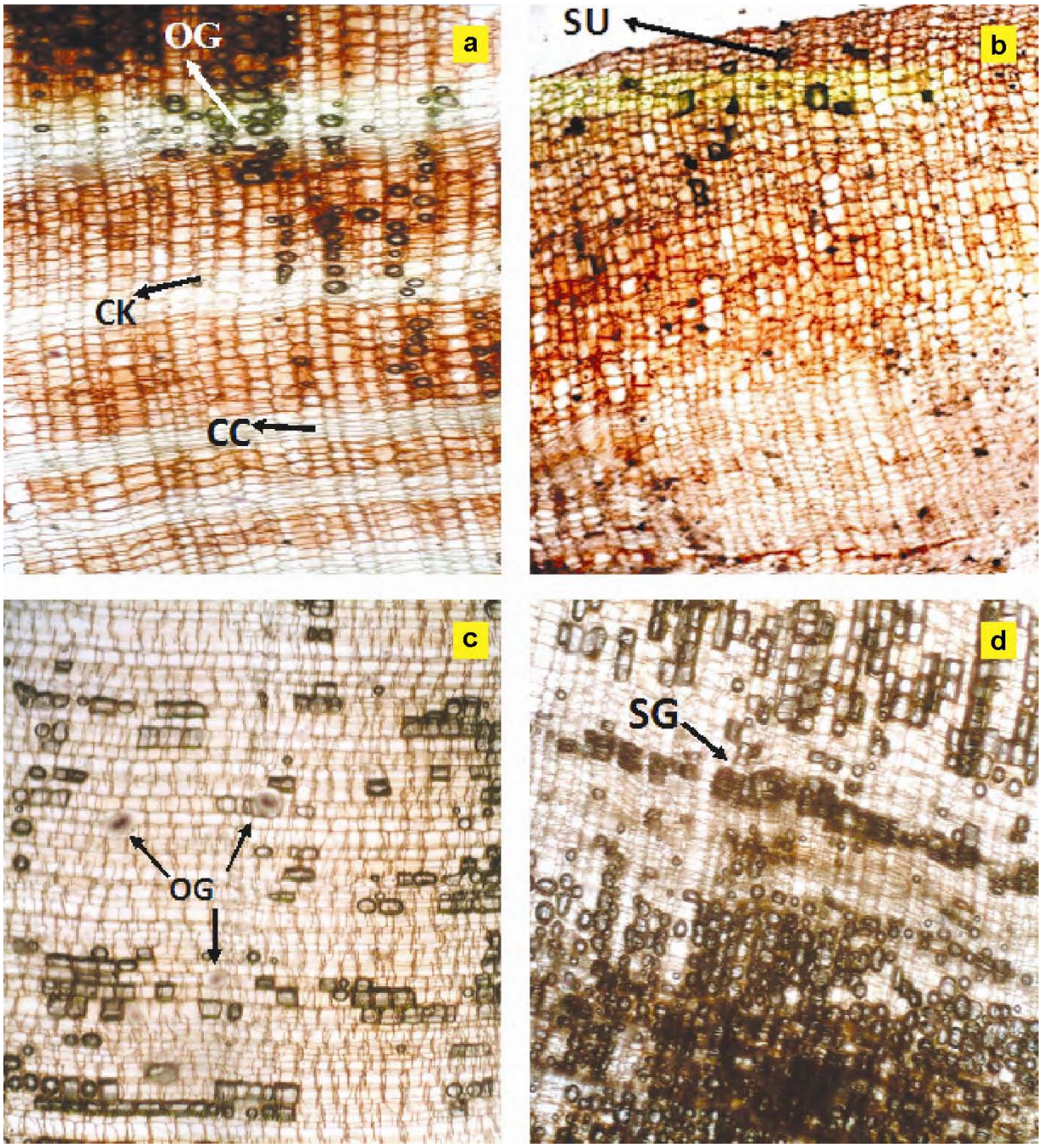

Plate 1—Transverse section of the bark of *Moringa oleifera* Lam. (a.OG-Oil globules, CK-Cork cells and CC- Cork cambium; b. SU-Suberised cells; c. OG-Oil glands; d. SG-Starch grains).

oil globules. The results of histochemical tests are shown in Table 1.

Diagnostic characters of powder

The powder is light brown in colour with characteristic odour and no taste. Under microscopic observation the powder showed thin walled hexagonal and polygonal cork cells either in groups or single, calcium oxalate crystals, starch grains, oil globules and phloem fibers (Plate 2). Behavior of the powder with different chemical reagent is given in Table 2.

Physico-chemical analysis

Ash values of the drug gave an idea of the earthy matter or the inorganic composition and other

Table 1—Histochemical tests on transverse sections of the bark of *Moringa oleifera* Lam.

Reagent	Observations	Constituent	Tissue
Pholoroglucinol + hydrochloric acid	No pink colour	Lignin absent	Periderm, Cortex
Aniline sulphate + sulphuric acid	No yellow colour	Lignin absent	Periderm, Cortex
Conc. Sulphuric acid	Green colour	Cellulose present	Cortex
Sudan III solution	Red colour	Oil globules present	Cortex
Aqueous ferric chloride	Black colour	Tannins present	Cortex
Dragendorff's reagent	No light orange colour	Alkaloids absent	Cortex
Ruthenium red	No pink colour	Mucilage absent	Cortex
Weak Iodine solution	Blue colour	Starch present	Cortex
Caustic alkali + hydrochloric acid	Green colour	Calcium oxalate present	Cortex

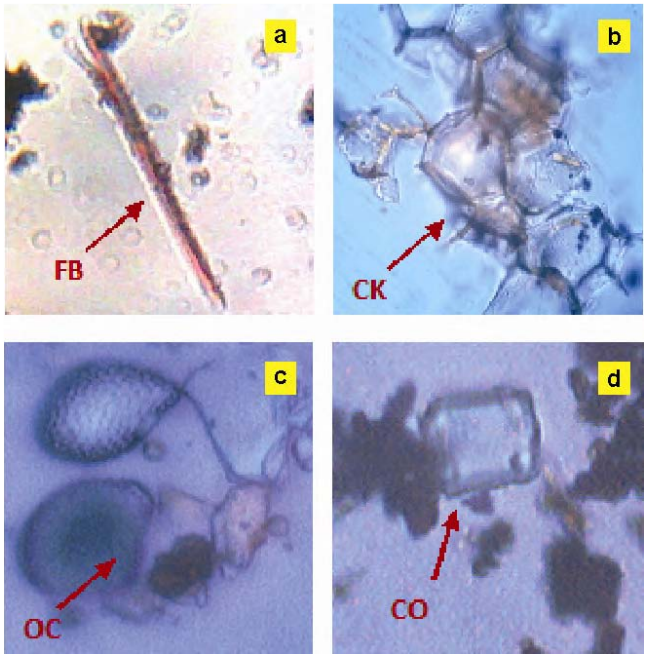

Plate 2—Powder characteristics of the bark of *Moringa oleifera* Lam. (a.FB-Pericyclic fibres; b. CK-Cork cells; c. OC-Oil Cell; d. CO-Calcium oxalate crystal)

impurities present along with the drug. The extractive values are primarily useful for the determination of exhausted or adulterated drug. The results of physico-chemical analysis of the bark of *M. oleifera* Lam. is presented in Table 3.

Fluorescence analysis

Powdered drug was treated with different reagents and was examined under day light and UV light (254 & 366 nm) the results are shown in Table 4.

Preliminary phytochemical studies

Preliminary phytochemical studies indicated the presence of carbohydrates, isothiocynate glycosides, triterpenoids and tannins in the ethanolic extract where as petroleum ether extract exhibited the presence of steroids only (Table 5).

Table 2—Behaviour of bark powder of *Moringa oleifera* Lam. with different chemical reagents

Reagent	Observation	Constituent
Aqueous ferric chloride (5%)	Black colour	Tannins present
Iodine solution	Blue colour	Starch present
Dragendorff's reagent	No reddish brown ppt	Alkaloids absent
Magnesium-hydrochoric acid	No change	Flavonoids absent
Ammonical solution	No change	Anthraquinone glycosides absent
Libermann-Burchard reagent	Green colour	Steroids present
Silver nitrate solution	Black colour	Isothiocynate glycosides present

Table 3—Physico-chemical analysis of the bark of *Moringa oleifera* Lam.

Parameter	Percentage w/w
Total ash	9
Acid insoluble ash	1.5
Water soluble ash	2
Sulphated ash	12
Loss on drying	6.5%
Ethanol extractive (cold maceration)	7.2%
Petroleum-ether extractive (cold maceration)	1.6%
Ethanol extractive (hot extraction)	10.4%
Petroleum-ether extractive (hot extraction)	2.3%

TLC finger print profile

The thin layered chromatographic study of the petroleum ether extract revealed 6 spots with Rf values in the range of 0.06 to 0.91 and the ethanolic extract revealed 5 spots with Rf values in the range of 0.10 to 0.77 (Table 6).

Table 4—Fluorescence analysis of powdered bark of *Moringa oleifera* Lam.

Reagent	Visible light	UV light	
		Short Wave (254 nm)	Long Wave (365 nm)
Powder as such	Brown	Light brown	Light brown
Powder + 1N Sulphuric acid	Orange	Yellowish green	Brown
Powder + 1N Nitric acid	Orange	Green	Green
Powder + Ammonia	Dark brown	Greenish brown	Brown
Powder + 1N HCl	Chocolate brown	Greenish brown	Dark brown
Powder + 1N Aqueous NaOH	Dark brown	Greenish brown	Dark brown
Powder + 1 N Alcoholic NaOH	Dark brown	Greenish brown	Dark brown

Table 5—Preliminary phytochemical studies of the bark of *Moringa oleifera* Lam.

Tests	Ethanolic Extracts	Petroleum Ether extract	Tests	Ethanolic Extracts	Petroleum Ether extract
Test for carbohydrates			**Tests for anthraquinone glycosides:**		
Molish's test (General test)	+	-	Borntrager's test	-	-
Fehling's test (Test for reducing sugars)	+	-	Modified Borntrager's test	-	-
Benedicts test (Test for reducing sugars)	+	-	**Tests for saponin glycosides**		
Barfoeds test (Test for Monosaccharides)	+	-	Foam test	-	-
Test for proteins			**Tests for coumarin glycosides**	-	-
Biuret test (General test)	-	-	**Test for cynogenetic glycoside**	-	-
Million's test (General test)	-	-	**Test for isothiocynate glycosides**	+	-
Test for steroids			**Tests for flavonoids**		
Salkowski test	-	+	Shinoda test	-	-
Liebermann–Burchard test	-	+	**Tests for tannins and phenolic compounds**		
Test for triterpenoids			Alkaline reagent test	-	-
Salkowski test	-	+	Ferric chloride test	+	-
Liebermann–Burchard test	-	+	Lead acetate test	+	-
Test for glycosides			Gelatin test	+	-
General test (hydrolysis test)	+	-	**Tests for alkaloids**		
Tests for cardiac glycosides:			Dragendroff's test:	-	-
Baljet's test	-	-	Mayers test	-	-
Legal's test (For cardenoloids)	-	-	Hagers test	-	-
Test for deoxysugars (Kellar Killani test)	-	-	Wagners test	-	-

Table 6—TLC fingerprints of ethanolic and petroleum ether extract of stem bark of *Moringa oleifera* Lam.

Number of spots	Ethanolic extract	Petroleum ether extract
	Rf values	Rf values
1	0.10	0.06
2	0.31	0.13
3	0.53	0.34
4	0.64	0.64
5	0.77	0.73
6	-	0.91

Conclusion

The pharmacognostic and physico-chemical parameters can be used for judging the adulteration and purity of this drug. The diagnostic features have been established to identify *M. oleifera* Lam. bark. Some of the diagnostic features of the bark are the characteristic odour and presence of cork cells. The bark has shown the presence of terpenoids and glycosides. TLC profiles helps in identification of various phytochemcial constituents present in the crude drug thereby substantiating and authenticating

of crude drug. The TLC profile also helps to identify and isolate important phyto-constituents. These finding could be helpful in identification and authentication.

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