Pharmacognostical & Phytochemical Evaluation of Root of *Mangifera indica* Linn.

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Summary

Mangifera indica Linn (Anacardiaceae) is well known for curing a variety of ailments such as abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths. The present study was undertaken to investigate the Pharmacognostical and Phytochemical parameters of stem of *Mangifera indica*. Pharmacognostical investigations were carried out to study its macro and microscopical characters. Various physiochemical parameters and histochemical color reactions were evaluated as per the IP method. The transverse section of the root revealed the presence of cork cells, phelloderm, phloem, xylem, medullary ray, pith etc. The results of physiochemical parameters showed total ash- 3.44% w/w, acid insoluble ash- 0.99% w/w, sulphated ash- 3.542% w/w. Petroleum ether soluble extractive- 0.28% w/w, ethyl acetate extractive- 13.9% w/w, chloroform extractive- 13.78% w/w, ethanol extractive-0.94% w/w, water soluble extractive-5.74% w/w and moisture content- 11.7% w/w. The qualitative evaluation of the extract indicated the presence of alkaloids, saponins, amino acids carbohydrates, glycosides, sterols, flavonoids, phenolic and tannins. Foreign organic matter, swelling index and crude fibre content were found to be 0.9% w/w, 0.23ml/mg and 5.55% w/w respectively.

Keywords: Anacardiaceae*, Mangifera indica*, Pharmacognostical, Phytochemical

Introduction

Mangifera indica Linn (Anacardiaceae) commonly known as mango, chosa, am, is native to southern Asia, especially Burma and eastern India. It spread early on to Malaya, eastern Asia and eastern Africa. Mangos were introduced to California (Santa Barbara) in 1880. In this day and age, *M. indica* resides in most tropical biotopes in India, Southeast Asia, Malaysia, Himalayan regions; Sri lanka, Africa, America and Australia [1-3]. Mangos basically require a frost-free climate. The mango must have warm, dry weather to set fruit. The plant is used in ophthalmia and eruption, hemorrhage of uterus, lungs or intestine. The ripe fruit is laxative, diuretic. The dried mango peel can be used as a fuel for biogas plant. The all parts are used to treat abscesses, broken horn, tumour, snakebite, stings, datura poisoning, heat stroke, anthrax, blisters and wounds in the mouths. The seed kernel extracts have antibacterial activity against *Bacillus subtilis, Staphylococcus albus and Vibrio cholerae* [4 - 6] and antifungal activity [5]. An alcoholic extract of the seed kernel of *Mangifera indica* has anti-inflammatory activity [7]. Mangiferin was found to be effective in controlling herpes simplex virus type 2, in vitro [8, 9]. The induction of interferon release from the macrophages. The mangiferin have Immunomodulatory activity [9 - 11]. A 50% ethanolic extract of the leaves has hypoglycemic activity.

The most abundant terpene hydrocarbons in fruits are limonene, β- myrcene and cis and trans- ocimene and most abundant oxygenated compound include methyl butanoate, ethyl 2 methyl butanoate, α- terpineol. The fruit pulp contains vitamins A and C, β–carotene and xanthophylls. An unusual fatty acid cis 9, cis 15- octadecadienoic acid (mangiferic acid, 5.4% of total acyl groups) is present in the pulp lipids of mango fruit from philipines, whereas a common octadecadienoic acid, linoleic acid [12], is in minor quantity. The leaves contain a petacyclin triterpene alcohol, indicenol [13], besides taraxone, taraxerol, fridelin, lupeol and β- sitosterol[14].Mango leaves contain several sugars and amino acids. Some esters of benzophenone glycosides and kinic and shikmic acids has also been reported from the leaves [15]. The leaf and flower yield an essential oil containing humulene, elemene, ocimene, linalool [16], camphene [17], nerol. The stem bark contains the mangiferin [18] and triterpens mangophanol (nopan-28-al-mangoleanone (olcanan-3-one) and mangiferolic acid, dihydro mangiferolic acid, mangiferonic acid [19], 5α stigmastane-3β-6α-diol. Indicoside A and B, manghopanal, mangoleanone, taraxerol, friedelin, cycloaratan-3 beta-30-diol and derivatives, mangsterol, manlupenone, mangocoumarin, n-tetacosane, n-heneicosane, n-triacontane and mangiferolic acid methyl ester and Mangostin, 29-hydroxymangiferonic acid and mangiferin have been isolated from the stem bark of *mangifera indica*. The flowers yielded alkyl gallates such as gallic acid, ethyl gallate, methyl gallate, n-propylgallate, n-pentyl gallate, n-octyl gallate, 4-phenyl-nbutylgallate, 6-phenyl-n-hexyl gallate and dihydrogallic acid. The roots contains the chromones, 3–hydroxy-2-(4'-methylbenzoyl)-chromone and 3-methoxy-2-(4'-methylbenzoyl)-chromone**.**

As per the available literature no Pharmacognostical study has been carried out on the root; hence the present investigation was undertaken to evaluate various Pharmacognostical standards like macroscopy and microscopy of root; ash values, extractive values, microscopical characteristics of powdered root and preliminary Phytochemical analysis of *Mangifera indica* Linn root.

Material and Methods

Collection of plant material: The root of *Mangifera indica* Linn was collected freshly from Sonepat (Haryana) in the month of December 2008 depending upon its easy availability. It was authenticated by Dr. H.B. Singh, at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (letter no. NISCAIR/RHMD/Conslt/2008-09/1121/152). The root of *Mangifera indica* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

Chemicals and instruments: Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a Labomed ATC-2000 microscope attached with Sony camera. Solvents used for extraction includes viz. petroleum ether, chloroform, ethyl acetate, ethanol (95%), water and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from Central Drug House (P) Ltd., New Delhi, India.

Macroscopic and Microscopic analysis: The macroscopy and microscopy of the root and powder were studied according to the method of Brain and turner [20]. For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen [21]. The microscopic analysis of powder was done according to the method of Brain and Turner [22] and Kokate [23].

Physico-chemical analysis: Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed [24) and the WHO guidelines on the quality control methods for medicinal plant materials [25]. Fluorescence analysis was carried out according to the method of Chase and Pratt [26] and Kokoski et al. [27].

Preliminary phytochemical screening: Preliminary phytochemical screening was carried out by using standard procedures described by Kokate [28] and Harborne [29].
The shade dried and powdered root of *Mangifera indica* Linn was subjected to maceration with different solvents like petroleum ether (60-80^{0}C), chloroform, ethyl acetate, ethanol and finally macerated with water so as to get respective extracts. All extracts were filtered individually, evaporated to dryness. After drying, the respective extracts were weighed and percentage yields were determined separately and stored in freeze condition for further use. The qualitative chemical tests, for identifying the presence of various Phytoconstituents, were carried out on various extracts of *Mangifera indica* Linn root.

Results and Discussion

Pharmacognostical Studies:

Macroscopic characters of the plant – *Mangifera indica* is a large spreading evergreen tree distributed through out in India, in forests up to 1200m altitude also widely cultivated. It is a large evergreen tree, long living, 10-45m high, with dense rounded or globular crown. The tree is long with some specimens known to be over 300 years old and still fruiting. The yellowish or reddish flowers are borne in inflorescences which appear at branch terminals, in dense panicles of up to 2000 minute flowers. Mangos are monocots and self-fertile, so a single tree will produce fruit without cross pollination. Polyembryonic types may not require pollination at all. The fruits are 2 to 9 inches long and may be kidney shaped, ovate or (rarely) round. The leathery skin is waxy and smooth, and when ripe entirely pale green or yellow marked with red, according to cultivar. The flesh of a mango is peach like and juicy, with more or less numerous fibers radiating from the husk of the single large kidney-shaped seed. The flavor is pleasant and rich and high in sugars and acid. The seed may either have a single embryo, producing one seedling, or polyembryonic, producing several seedlings that are identical but not always true to the parent type the morphological studies revealed that the root was erect, woody, profusely branched, cylindrical, glabrous, solid and yellowish brown in color with no odour and taste.

Microscopic characters:

Transverse section of stem – The root is almost circular in cross-sectional view consisting of stratified cell in altering bands known as cork cells and outermost layer is Phelloderm having a single layer of tangentially elongated parenchymatous cells, covered with a thick cuticle layer containing brownish matter.
The vascular tissues consist of primary phloem, secondary phloem, cambium, primary xylem, secondary xylem. Secondary xylem forms the largest zone. Medullary rays are present in the vascular tissue and these are parenchymatous 2 – 3 cells thick. Between the different layers of phloem medullary rays are also present. The central pith is filled with thin walled parenchymatous cells [Fig. 1].

***Histochemical color reactions*-** The Histochemical color reactions on the root were performed for the identification of major cell components. For color tests transverse sections of fresh root were treated with different chemical reagents viz. Millon's reagent, Iodine

solution, Wagner's reagent, Dragendorff's reagent, $FeCl_3$ solution, Dilute H_2SO_4, Chloroform & dilute H_2SO_4. The changes in the histochemical zones were observed under microscope and the results are shown in Table 1**.**

Powder characters - The root powder is yellowish brown in color. On microscopical examination the powder showed reticular tracheids [Fig. 2] and pericyclic fibres [Fig. 3]. scleranchyma cells [Fig. 4] was seen in powder microscopy. The medullary rays [Fig. 5] were clearly shown in the slide. Starch grains having a small diameter were also observed [Fig. 6].

Color reactions of root powder- To study the behavior of root powder with different chemical reagents, the powder was treated with different chemical reagents viz. 1N- NaOH, 1N-HCl, acetic acid, picric acid, 5% ferric chloride, 1N- HNO_3, 5% iodine and 1N- HNO_3 followed by ammonia solution and colors were observed. The results are shown are shown in Table 2.

Physico-chemical studies – Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash, sulphated ash and water soluble ash were carried out. The results are shown in Table 3. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble, alcohol soluble and ether soluble extractive values have been tabulated in Table 4. The results of fluorescence analysis of the drug powder are presented in Table 5.

Preliminary phytochemical screening – Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, tannins, triterpenoids, carbohydrates and flavonoids. The results are shown in Table 6.

Table 1: Histochemical color reactions of stem (TS)

S. No.	Reagents	Test for	Color change	Degree of change
1.	Millon's reagent	Proteins	Yellowish red	-
2.	Iodine solution	Starch	Black	+
3.	Wagner's reagent	Alkaloids	Brownish green	-
4.	Hagner's reagent	Alkaloids	Brown	-
5.	$FeCl_3$ solution	Tannins	Greenish black	+
6.	Dilute H_2SO_4	sterols	Blackish brown	-
7.	Chloroform + dilute H_2SO_4	sterols	Blackish brown	-

- No color change, + Color change

Table 2: Behavior of stem powder with different chemical reagents

S. No.	Treatment	Color
1.	Powder	Yellowish brown
2.	Powder + 1N- HCl	Light yellow
3.	Powder + 1N- NaOH	Reddish brown
4.	Powder + Acetic acid	Yellow
5.	Powder + 5% Ferric chloride	Light yellow
6.	Powder + Picric acid	Yellow
7.	Powder + 5% Iodine	Reddish brown
8.	Powder + 50% HNO_3	Red
9.	Powder + 50% H_2SO_4	Blackish brown

Table 3: Ash values of stem powder

S. No.	Ash values	% w/w
1.	Total ash	3.44%
2.	Acid insoluble	0.99%
3.	Sulphated ash	3.542%

Table 4: Percentage extractive values of stem

S. No.	Solvent	Color	Average extractive value (w/w)
1	Petroleum ether (60-80^o)	Light yellow	0.28%
2	Chloroform	Yellow brown	13.78%
3	Ethyl acetate	Yellow	13.9%
4	Ethanol	Yellow	0.94%
5	Water	Brown	5.74%

Table 5: Fluorescent nature of stem powder

S. No.	Treatment	Observations		
		Long UV	Short UV	Visible
1.	Powder as such	Dark brown	Green	Brown
2.	Powder + 1N HCl	Light brown	Green	Brown
3.	Powder + 1N NaOH	Dark brown	Green	Brown
4.	Powder + 50% HNO_3	Light brown	Greenish black	Light brown
5.	Powder + 50% H_2SO_4	Dark brown	Greenish brown	Brown
6.	Powder + Methanol	Dark brown	Dark green	Brown
7.	Powder + Acetic acid	Dark brown	Greenish brown	Brown
8.	Powder + picric acid	Dark brown	Yellowish brown	Yellowish brown
9.	Powder + 5% Iodine	Red	Reddish brown	Reddish brown

Table 6: Preliminary phytochemical screening

S. No.	Test for	P. Eth (60-80°)	$CHCl_3$	Ethyl acetate	Ethanol	Water
1	Alkaloids	-	-	-	-	-
2	Carbohydrates	-	+	+	+	+
3	Glycosides	-	-	+	+	+
4	Sterols	-	+	+	+	-
5	Saponins	-	-	-	-	-
6	Phenolic comp. & tannins	-	-	-	+	+
7	Proteins	-	-	-	+	+
8	Free amino acids	-	-	-	+	+
9	Flavonoids	-	+	+	+	+

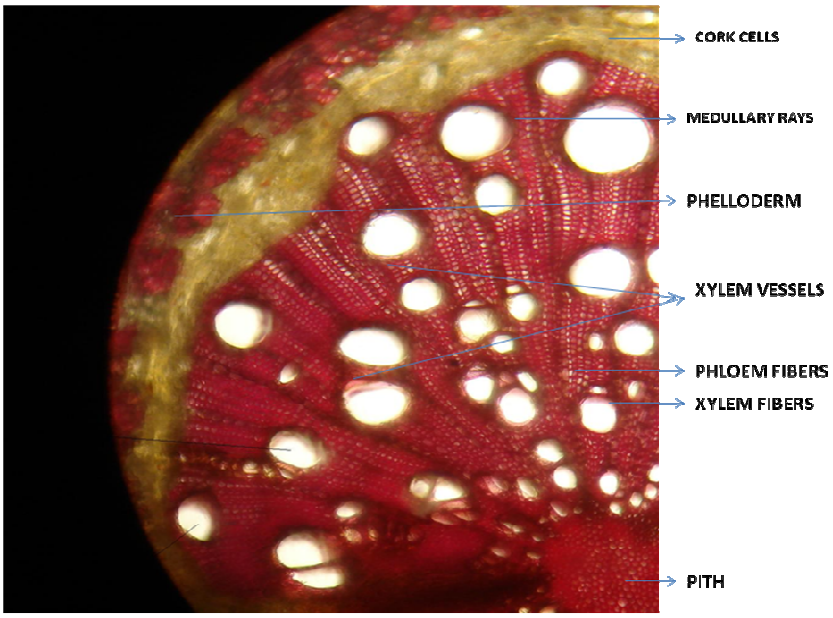

Fig. 1: The TS of root of *mangifera indica*

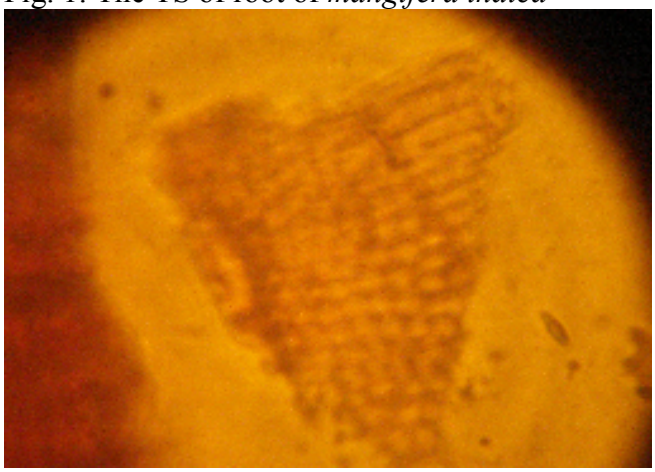

Fig.2: Reticular tracheids

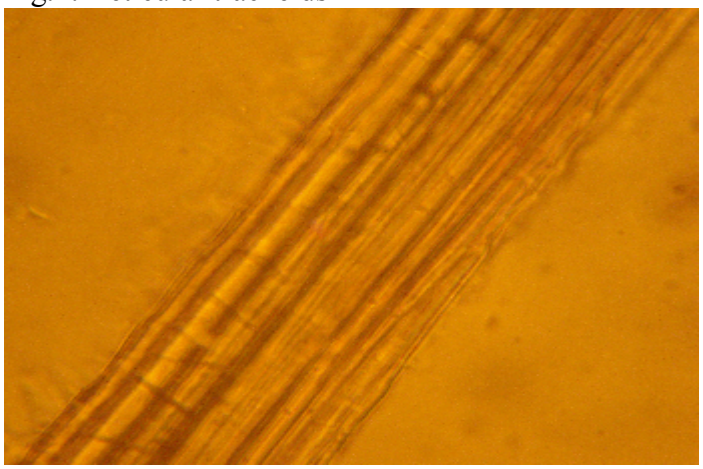

Fig. 3: Pericyclic fibres in surface view

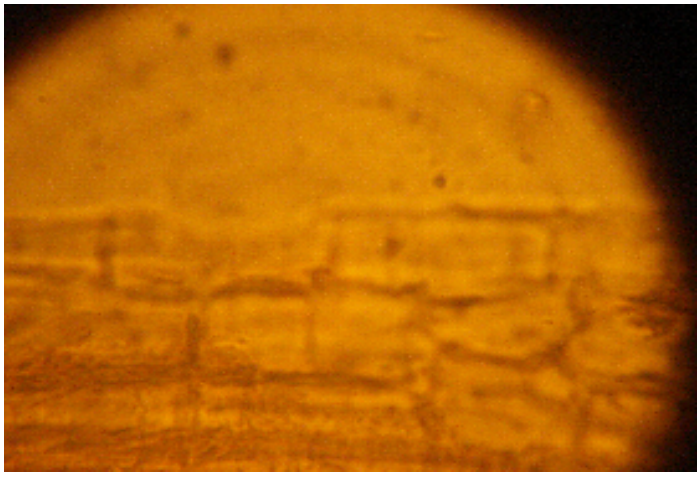

Fig. 4: Scleranchyma cells

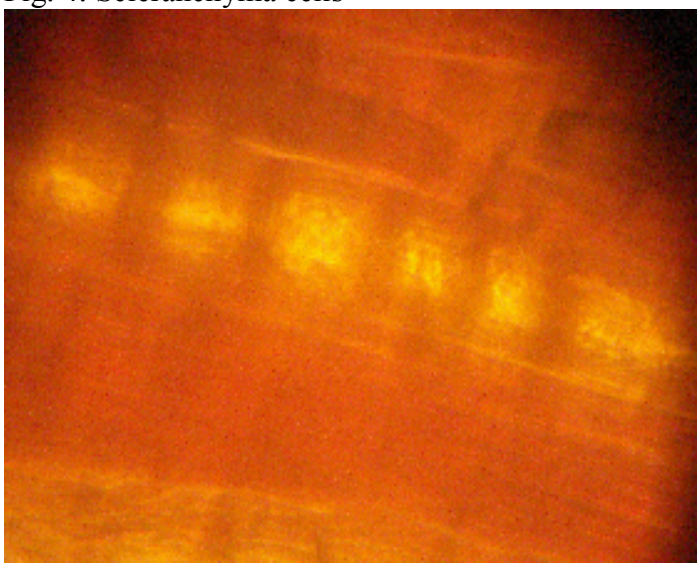

Fig. 5: Medullary rays

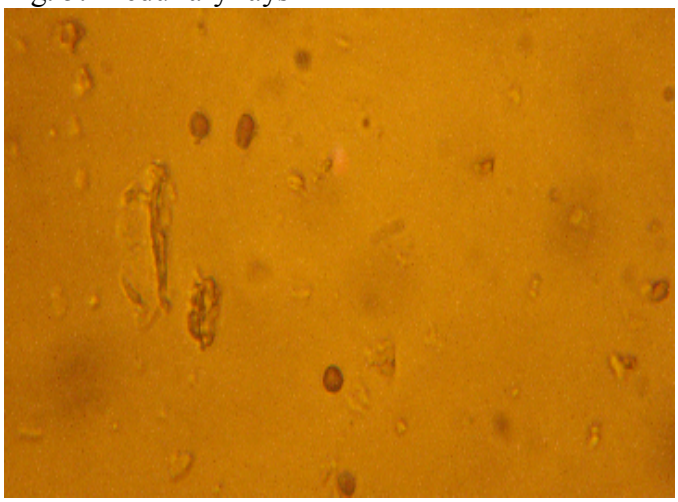

Fig. 6: Starch grains having a small diameter

Conclusion

The present study on Pharmacognostical & Phytochemical evaluation of *Mangifera indica* will provide useful information for its identification. Macro, micro and physiochemical standards discussed above can be considered as the identifying parameters to substantiate and authenticate the drug.

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