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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF TENDER AND MATURE LEAVES OF *MANGIFERA INDICA* LINN.

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ABSTRACT

Amrapallava, which is identified as tender leaves of mango tree (Mangifera indica Linn) are used for various ailments in Ayurvedic system of medicine since ancient time. But there is no scientific data is available on different maturity levels of leaves for therapeutic use. Hence the present study compared the Pharmacognostical and Phytochemical standards of the leaves of Mangifera indica Linn at different maturity. Macroscopic, microscopic, physicochemical, phytochemical, TLC, HPTLC and AAS tests were done as per the standard procedure described in Ayurveda Pharmacopoeia of India. Among the macroscopic characteristics, the colour of tender leaves of Mangifera indica Linn is pinkish, amber or pale green colour while that of mature leaves having dark green colour. Texture of tender and mature leaves of Mangifera indica Linn was thin leathery and coriacious respectively. In transverse section the resin canal was more in mature leaves of mango tree. Tetrahedral prismatic crystals are present in tender leaves which is absent in mature leaves. The percentage of water soluble extractive and fibre content was comparatively more in mature leaves. Steroids were absent in both tender and mature leaves. Percentage of Phenolic content was more in mature leaves, and there is no other marked variation in all other parameters.

KEYWORDS: *Mangifera indica* Linn, Pharmacognosy, Phytochemistry, *Amra.*

INTRODUCTION

Amrapallava, which is identified as tender leaves of mango tree (Mangifera indica Linn) are used for various ailments in ayurvedic system of medicine since ancient time. Mangifera indica Linn belongs to the family Anacardiaceae. Mangifera indica Linn is a large spreading evergreen tree found throughout India, except in the temperate regions, both in the wild and mostly under cultivation. The leaves are perennial, crowded at the end of branches, Simple, coriaceous, oblong, oblong- lanceolate, deep green when mature and tender leaves having pink ,amber or pale colour.[1] Mango gives flushes of new growth from October/November depending on climatic condition.[2]

According to Ayurveda, varied medicinal properties are attributed to different parts of mango tree. Different parts of this tree are traditionally used for various medicinal purposes. In Charaka samhitha *Amrapallava* is mentioned in chardinigraha gana ^[3] (group of anti-emetic drug). Kaiyyadeva nighantu (lexicon of Ayurveda) included *Amrapallava* in *panchapallava* (5 types of tender leaves). ^[4] In Bhavaprakasha nighantu(lexicon of Ayurveda) it is mentioned that *Amrapallava* is having *Kashaya rasa*

(Astringent taste) *Kaphapitta hara karma* (Pacifies *Kapha* and *Pitta dosha*). [5]

Amrapallava is used for diarrhoea, Vomiting, fever etc. Mature leaves are also used for toning up the gums, inflammation of eye, burns, scalds, cuts^[6] etc. As per Sharangadhara samhitha-Swarasa (juice) of tender leaves of Jambu (Syzigium cumini), amra (Mangifera indica), Amalaka (Emblica officinale) taken with honey, ghee and milk relieves even severe raktatisara (bloody diarrhoea).^[7] In Chikitsamanjari (a traditional textbook of Kerala) it is mentioned that paste of Amrapallava along with tender coconut water is given for kamala (hepatobiliary diseases).^[8]

Mangifera indica Linn is commonly used in folk medicine for a wide variety of remedies like diabetes, hiccup, throat affections, digestive problems, stones etc. The leaves are reported to possess antibacterial activity^[9], antiulcerogenic action^[10], hypoglycemic activity^[11], atherogenicity^[12], antioxidant^[13] and hepatoprotective activity.^[13] There is no idea regarding the maturity of mango leaves to be used for various ailments. From the present study, it can be evaluated whether mature leaves can be used instead of tender leaves of Mangifera indica as the later is not available in all seasons. Pharmacognosy

and phytochemistry of mature leaves of mango tree was already done by Jalalpure SS and et al.^[14] Scarce data is available on pharmacognosy and phytochemistry of tender leaves of mango tree.

In short, there are good level of traditional and experimental evidences to support various claims and advantages of the leaves of this widely available plant. As mentioned earlier several reports have been published on the effects of the leaves on different biological activities *in vitro* and *in vivo*. An investigation regarding scientific data on maturity of the leaves of *Mangifera indica* Linn is inevitable. Hence, it is an attempt to evaluate changes in microscopic, physicochemical and phytochemical characteristics of the mature and tender leaves of the plant.

MATERIALS AND METHODS Collection of Plant material

The tender and mature leaves of *Mangifera indica* Linn were collected for the study from same area of Kollam district. For the present study the fresh drug was taken and it was later dried in shade. After drying it was pounded to coarse powder. Powdered drug was stored in an air tight container.



Figure no.1 Tender and mature leaves of Mangifera indica Linn

a. Pharmacognostical Evaluation Macroscopic Evaluation

Macroscopic and organoleptic studies were conducted on intact materials. Sample was washed, air dried in shade and observed for colour, shape, odour, taste, and other surface characteristics. A magnifying lens was used for a better evaluation of surface characters.

Microscopic evaluation

The microscopic evaluation of sample drug includes histological evaluation and powder microscopy were done as per the standard procedures.^[15] Cross-sections were prepared by free hand sectioning and stained with freshly prepared dye safranin. All the images presented were taken by a digital microscope (Olympus Student's microscope with CCD camera).

b. Physicochemical evaluation

The preliminary physicochemical and phytochemical analysis of the genuine sample of tender and mature leaves of *Mangifera indica* Linn were done according to the standard procedures described in API (Ayurvedic Pharmacopoeia of India). It includes: -Various parameters like foreign matter, moisture content, volatile oil content, ash values, fiber content, sugar content, different extractive values, qualitative analysis, heavy metal analysis by Atomic Absorption Spectroscopy and chromatographic techniques like TLC, HPTLC etc.

Reagents used

Xylene, dilute Hydrochloric acid, Petroleum ether, Cyclohexane, Acetone, Acetic anhydride, concentrated Hydrochloric acid, concentrated Sulphuric acid, Magnesium ribbon, neutral Ferric chloride, Benzene, Chloroform, Ethyl acetate, Potassium permanganate, Acetic acid, Fehling's solution, Sodium bicarbonate, Dragendroff's reagent, Ferric alum, Ethanol, Lead acetate, Sodium oxalate, Ethanol, and distilled water.

Apparatus

Dean and Stark's apparatus, Clevenger apparatus, Soxhlet apparatus, silica crucible, Bunsen burner, round bottomed flask, measuring jars, beakers, conical flask, funnel, glass rods, watch glass, electronic balance etc.

RESULTS AND DISCUSSION

I. Results of macroscopic evaluation

Mangifera indica Linn. is a large spreading evergreen tree. The organoleptic characteristics of mature and tender leaves of fresh plant of Mangifera indica Linn were summarized in Table No: 1

II. Results of microscopic evaluation

Transverse section of Mature and tender leaves of *Mangifera indica* Linn. (Fig:2)

The transverse section of tender and mature leaves of *Mangifera indica Linn*. shows lamina and midrib regions. The lamina consists of upper palisade and lower spongy parenchyma cells. Midrib showed centrally located vascular bundle. Detailed transverse section showed upper and lower single layered compactly arranged barrel shaped epidermal cells with cuticle. Mesophyll consists of 1-2 layers of upper compactly arranged elongated palisade and lower 5-6 layers of oval to rounded shaped spongy parenchyma cells were rich in chlorophyll pigment. Between the palisade and spongy parenchyma vascular strands are passing through all over the lamina. Lamina portion interrupted by resinous canals contain oil and resin.

Transverse section through midrib showed that both the epidermis consists of 6-8 layers of

collenchyma followed by circularly arranged 3-5 layered pericyclic fibers. Ground tissue made up of parenchyma cells 4-5 large resinous canals/cavities covering the vascular bundle. Ground tissue made up of parenchyma cell consists of tetrahedral prismatic crystal of calcium oxalate, tannin content and oil globules, scattered all over the tissue and inside the cavity also. In mature leaves, ground tissue made up of parenchyma cell contains prismatic crystal and interrupted by 8-10 large resin canals covering the vascular bundle. Centrally, located parenchymatous cells completely surrounded by vascular bundle. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma, and its fiber.

The powder microscopy of mature and tender leaves of *Mangifera indica* Linn.

The powder prepared by tender and mature leaves of *Mangifera indica* Linn. was screened for the presence of various microscopic features.

Tender and mature leaves of *Mangifera indica* Linn. showed -Cluster, prismatic crystal, wavy parenchyma cell, fibre, Stomata, pitted vessels shown in fig no: 3

III. Results of physico-chemical evaluation

Preliminary physical and physico-chemical analysis were done and the results are summarized in Table No: 2. These procedures give valuable information about the identity, purity and genuineness of the drug. The ash values of a drug give an idea of the inorganic composition and other impurities present along with the drug and the value was more in mature leaf (1.2%) than tender leaves (1.4%). Estimation of extractive values determines the amount of the active constituents in a given plant material when extracted with a particular solvent. The high water solubility of the contents than alcohol

solubility of both tender and mature leaves suggests use of aqueous extract for future studies.

IV. Results of preliminary phytochemical analysis

The ethanolic extractive obtained was subjected to qualitative analysis for identification of various plant constituents like steroids, phenols, alkaloids, flavonoids, tannins and results were summarized in Table No: 3. The presence of different plant constituents determines the pharmacological action and therapeutic potential of that plant. The preliminary phytochemical screening of both mature and tender leaves showed the presence of different phyto constituent groups such as, flavanoid, phenol, alkaloid and tannin and the steroid was absent in both mature and tender leaves.

V. Results of TLC and HPTLC

The spots obtained in TLC gives a rough idea about the study plant constituents. The best separation was achieved using Toluene: Ethyl acetate: Formic acid (7:3:1) for both tender and mature leaves of *Amra* (*Mangifera indica* Linn). The plates were first viewed through UV-fluorescence viewing cabinet (365nm) and the Rf values of the spots were noted (Table No: 4). Two peaks were obtained for Tender leaves of *Mangifera indica* Linn at Rf values 0.14 and 0.35. Seven peaks were obtained for mature leaves of *Mangifera indica* Linn. at Rf values 0.14, 0.19, 0.33, 0.40, 0.48, 0.55, and 0.65. HPTLC profile is shown in figure no.4

VI. Results of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy is used in the determination of heavy metal elements and some non-metal elements in atomic state and the results were shown in Table No: 5. Four heavy metals-copper, cadmium, iron and lead contents were found within permissible limits. Hence the drug is not contaminated by heavy metals and can be used safely for internal administration.

Table 1: Macrosco	onic evaluation	of tender and	d mature leav	es of <i>Manaifer</i>	<i>a indica</i> Linn
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Characters	Tender leaves	Mature leaves
Shape	Lanceolate-elliptical, pointed at both ends	Lanceolate-elliptical, pointed at both ends
Size	25cm long, 8cm wide or varying size	25cm long, 8cm wide
Margin	Entire	Entire
Apex	Pointed	Pointed
Surface	Smooth	Smooth
Colour	Pinkish, amber or pale green	Dark green
Texture	Thin leathery	Coriaceous
Odour	Characteristic	Characteristic
Taste	Acrid, Astringent	Acrid, Astringent

Table 2: Physico chemical analysis of tender and mature leaves of Mangifera indica Linn.

S.No	Experiment	Tender leaves of Mangifera indica Linn.	Mature leaves of Mangifera indica Linn.
1	Foreign matter (%)	Nil	Nil
2	Moisture content (%)	0.9%	0.6%
3	Volatile oil (%)	0.1%	0.1%
4	Total ash (%)	1.2%	1.4%
5	Acid insoluble ash (%)	8.0%	8.3%
6	Water soluble extractive (%)	10.02%	12.06%
7	Alcohol soluble extractive (%)	8.10%	8.62%
8	Fibre content (%)	8.96%	21.4%
9	Sugar content		
	Reducing sugar (%)	2.39%	3.09%
	Total sugar (%)	2.74%	3.08%

Table 3: Results of Qualitative chemical analysis of alcoholic extract of tender and mature leaves of *Mangifera indica* Linn.

S. No	Experiment		Mature leaves of <i>Mangifera</i> indica Linn.
1	Steroid	Ayurved	_
2	Flavanoid	+ and http://ijapr.in	+
3	Phenol	+3	++
4	Alkaloid	art)	+
5	Tannin	+++	+++
6	Saponin	+ 62	+

(+) for present, (-) for absent

Table 4: Rf values of spots obtained in Chromatography

Drug	Solvent system	No.of spots	Colour of spots	Rf value
Tender leaves of Mangifera indica Linn	Toluene: ethyl acetate: formic acid	2	Pink	0.65, 0.9
Mature leaves of Mangifera indica Linn	Toluene: ethyl acetate: formic acid	3	Pink+red	0.65, 0.9, 0.75

Table 5: Atomic absorption spectroscopy

S.No.	Heavy metal	Tender leaves of Mangifera indica Linn.	Mature leaves of Mangifera indica Linn.	Maximum permissible limits (ppm)
1.	Cadmium	0.0182	0.0160	0.3
2.	Lead	0.4084	0.4830	10
3.	Copper	0.3788	0.2250	10
4.	Iron	1.765	1.751	20

CONCLUSION

In the present study, pharmacognostic and phytochemical screening were carried out as per pharmacopoeia and WHO guidelines. The macroscopical and microscopical evaluations were done to ascertain the standard reference value for the

standardization of plant material. This study revealed that the phytochemical constituents may be responsible for various pharmacological activities of this medicinal plant. These results may help in standardization, identification and in carrying out further research in leaves of *Mangifera indica* Linn. Much difference was not noticed in the Pharmacognostical and Phytochemical characteristics of tender and mature leaves of *Mangifera indica* Linn.

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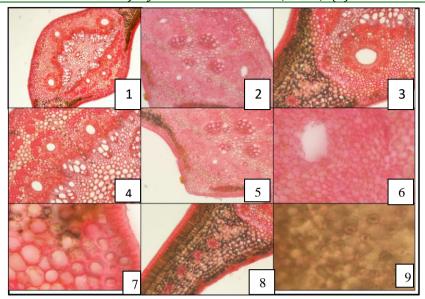


Fig No:2 Transverse section of tender and mature leaves of Mangifera indica Linn

1-Midrib of mature leaves 2-Midrib of tender leaves 3-Resin canal mature leaves 4-Vascular bundle of mature leaf 5-Vascular bundle of tender leaf 6-Resin canal of tender leaf 7-Prismatic calcium oxalate crystal 8- Lamina, 9-Stomata

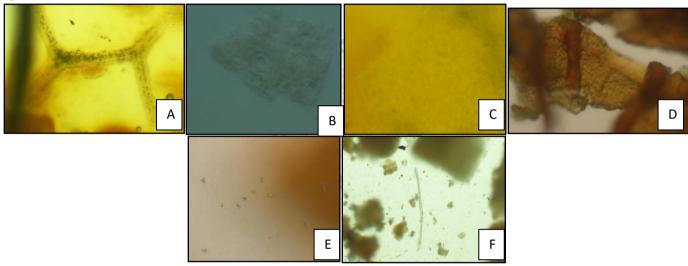


Fig No.3 Powder microscopy of tender and mature leaves of *Mangifera indica* Linn.

A-Prismatic crystals (Tender leaf), B-Parenchyma, C-Stomata, D-Pitted vessel, E-Prismatic crystal (Mature leaf), F-Fiber

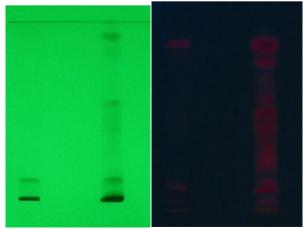


Fig no:4 HPTLC Plate with Toluene: Ethyl acetate: Formic acid in the ratio 7: 3:1(254 nm, 366nm), Track 1-Tender leaves of *Mangifera indica* Linn, Track 2- Mature leaves of *Mangifera indica* Linn.