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Research Article

Pharmacognostical and phyto-physicochemical investigation of *Mangifera indica* Linn.

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ABSTRACT

The aim of the present study is to describe the microscopically, physicochemical and phytochemical attributes of the *Mangifera indica* L. bark. This is required to generate preformulation data useful for the preparation of the pharmaceutical dosage form of *Mangifera indica* L. bark extract. Microscopic investigation was done using high magnification lens from Cerlzeis microscope. The physicochemical properties such as loss on drying, total ash, pH, solubility, extractive values etc. were determined on the bark extract powder. Phytochemical investigations such as qualitative phytochemical analysis were carried out as per standard method reported by T.E. Wallis. It was observed that the T.S. of matured bark has got distinct irregular cork, under which there are about 15-30 layers of rectangular tangentially elongated cells consisting of phellogen and pheloderm. There are stone cells scattered in the cortex Area. The results of present study revealed the presence of various active constituents such as Glycoside, Sterols, Saponins, Phenolic compounds, Tanins, Amino acids and Flavonoids. The investigational work suggested that *Mangifera indica* L. Hydroalcoholic dry extract contains the ingredients which can support the claim of the reported pharmacological activities of *Mangifera indica* L. aqueous extract (Vimang). Hence the present study helped and provided information required for as preformulation needed of *Mangifera indica* L. extract dosage form.

Keywords: *Mangifera indica* L. bark; Phytochemical screening; Pharmacognostical study

INTRODUCTION

Now-a-days there is a widespread interest in evaluating drugs derived from plant sources. This interest primarily stems from the belief that herbal medicine is safe and dependable, compared to synthetic drugs which are invariably associated with unwanted side effects (WHO., 2000). It has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Pandey et al., 2001). The use of traditional medicine has increased in developed countries also, mainly due to the failure of modern medicine to provide effective treatment for chronic diseases (Core FG and Anderson GJ., 1996). Plants are one of the prime sources of medicines and recently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, around 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine. The world is now focusing towards the herbal medicine or phytomedicines that repair and strengthens bodily systems (especially the immune system that can properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects.

Mangifera indica L. (Anacardiaceae) grows on the tropical and subtropical region and its parts are commonly used in Traditionl Medicine for a wide variety of ailments (Ansari M A et al., 1971). A standard aqueous extract of *Mangifera indica* L. is used in Cuba as an antioxidant under the brand name of Vimang and was tested *invivo* for its Anti-inflammatory activities (Garrido G et al., 2001). The aim of the present study was to investigate the diagnostic characteristic of *Mangifera indica* L. bark extract. In India the plant is abundantly grown in the hilly region of Maharashtra, Bihar and Tamilnadu etc. In Maharashtra the plant is found in Ratnagiriand Deogarh region. Various parts of the plant together or in isolation are used in the treatment of many ailments of the human being because of the presence of its antioxidant activity, radioprotective effect, antiallergic activity, antiinflammatory activity, antitumour activity and its immunomodulatory effect. These properties are mainly considered to be due to the presence of mangiferin in *Mangifera indica* L.

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India has a rich heritage of traditional medicine constituting with different system of medicine like Ayurveda, Siddha and Unani. The development of these traditional system of medicine with the perspective of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalise the use of natural product in healthcare (Kirtikar KR and Basu BD., 1993). *Mangifera indica* L. fruits are the important fruits in the world and India contributes major part of the world requirement. Bark and stem possesses immunomodulatory and neuroprotective activity (Sanchez GM et al., 2000).

The stem bark of *Mangifera indica* L. contains the mangiferin and triterpens mangophanol (nopan-28-almangoleanone (olcanan-3-one) and mangiferolic acid, dihydromangiferolic acid, mangiferonic acid, 5α stigmastane-3β-6α-diol. Indicoside A and B, manghopanal, mangoleanone, taraxerol, friedelin, cycloaratan-3 beta-30-diol and its derivatives, mangsterol, manlupenone, mangocoumarin, n-tetacosane, n-heneicosane, ntriacontane and mangiferolic acid methyl ester and Mangostin, 29-hydroxymangiferonic acid and mangiferin (Ross IA.,1999). As per the available literature practically no significant formulation development work has been carried out on *Mangifera indica* L. plant collectively or separately. The present investigation has generated useful information for the preformulation studies on the extract of *Mangifera indica* L. bark.

MATERIALS AND METHODS

Plant Collection and Extraction

The bark of *Mangifera indica* L. chosen for the present investigation was collected during the month of Nov. 2009, from different area of Maharashtra, India and were identified and authenticated by Dr. S. Rangdale, Prof. and Head, Department of Botany, Hon'ble B.J. College of Pharmacy, Ale, Pune University, and a voucher specimen was deposited in our department. The bark parts were separated, cleaned, air dried and grinded into powder. The powdered material was passed through sieve no 40, stored in air-tight container for further use.

EXPERIMENTAL

Preparation of extract

Air dried drug powder 500gm of *Mangifera indica* L. bark was extracted with Ethanol:Water mixture 80:20 for 24hours in a Soxhlet apparatus. The extract was filtered with muslin cloth and evaporated under reduced pressure and dried to constant weight. The dried extract was found to be 15% on dried basis and it contains 10% of mangiferin tested as per the method given in I.P. The extracts were mixed and kept in desiccators for further experiment. Physico-chemical analysis i.e. solubility, ash values and extractive values were determined according to the official methods (The Ayurvedic Pharmacopoeia. 2007).

Preformulation studies

Macroscopic characteristics

For morphological analysis, barks were used. The characteristic features were observed under magnifying lens (The Ayurvedic Pharmacopoeia. 2007).

Microscopic characteristics

Free hand section of the bark was taken and stained with safranine to confirm its lignifications (Tyler V et al., 1997).

Physicochemical Parameters

The physicochemical parameters like size and shape of the particles, ash values, total ash, acid insoluble ash, sulphated ash, loss on drying, water soluble ash value, acid insoluble ash value, solubility in petroleum ether, methanol, acetone and water soluble extractive values, pH value, flow properties of the powdered extract and weight loss of the material on heating were determined (Khandelwal KR., 2008; Rakholiya K. et al., 2012; Wagner H. and Bladt S. 1997; Mukherjee P.K., 2008).

Phytochemical analysis

The powder of *Mangifera indica* L. bark extract was subjected to qualitative phytochemical analysis (WHO., 2002; Harborne JB., 1973).

Thin Layer chromatography

The procedure used was as follows: For the stationary phase, fluorescent, precoated K5 silica plates were used and the details of the mobile phase are given in Table 5.

The analyte solution of 10µl each was applied from a microsyringe, as a band of 10mm by 2mm. The spot was allowed to dry before developing the plate. The level of the solvent in the developing tank was adjusted to a level 2 to 3 mm below the line of origin on the plate. The plate was considered developed when the distance between point of origin and the distance travelled by the solvent front was not less than ¾ of the length of the plate and no further than 5mm below the top of the plate. The plate was dried and sprayed with spraying reagent, examined the plate in day light. The retention factor, Rf value for a given spot were calculated dividing the distance travelled by each spot by the distance travelled by the solvent (Parekh J and Chanda S., 2007; WHO., 2007)

Microbiological studies

Mangifera indica Linn. Extract (10g) was suspended in 100ml of sterile peptone water. 1ml of peptone water suspension was placed in the 20ml sterile melted soya bean casein digest agar petridish in duplicate. The plates were incubated the plate at 30 ^{0} to 35^0 c for 5 days. The total aerobic bacterial counts were calculated by multiplying dilution factor to the mean number of CFU observed in incubated petridishes. The absence of E.coli, Salmonella, Shigella and candida albi-

cans, the method followed as given in I.P on 5gm of dried extract powder (Indian Pharmacopoeia., 2007).

RESULTS AND DISCUSSION

Identification

Macroscopic Characteristics

The dried stem bark pieces about 5-12cm long and 1.5 to 2.0cm thick were taken and observed for macroscopic characteristics. It was greyish white outside and dark brown inside, odourless and tasteless with rough outer surface uniform inner surface (Fig 1. and Fig 2).

Figure 1: Frontal surface of stem bark of *Mangifera indica* L

Figure 2: Dorsal surface of stem bark of *Mangiferaindica* L

Microscopic Characteristics

The matured bark showed about 15-30 layers of cork which were tangentially elongated, rectangular without any intracellular space. Outer cork cells were brownish in colour where as the intensity of colour decreases towards inside. Resin canals and yellow coloured tannins sacs were found in phloem region. Stone cells were thick walled and lignified prismatic, calcium oxalate crystals were present in cortical region (Fig. 3).

The transverse section (T.S) of the bark showed following characters

i) Outermost secondary cork consisting of transversely elongated, brown coloured cells.

ii) Phellogen containing 10-15 layers of tangentially elongated, thin walled parenchymatous cells.

iii) Phelloderm containing 10-15 layers of transeversely elongated, thin walled parenchymatous cells.

iv) Pericyclic region consisting of polygonal or rounded parenchymatous cells in which lignified, thick walled stone cells and lignified pericyclic fibres were disturbed.

v) Phloem region consisting of

a) Phloem parenchyma of rounded parenchymatous cells.

b) Phloem fibres slender, lignified and isolated.

c) Medullary rays made up of walled parenchymatous cells,3-4 celled, dividing the phloem tissue.

d) Clusters and isolated prismatic crystals of calcium oxalate in phloem parenchymatous cells.

e) Yellow coloured tannin tubes.

f) Resin Cells

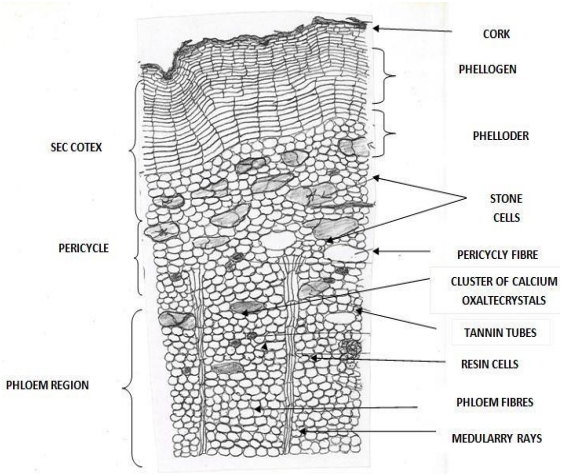

Figure 3: T.S OF *Mangifera indica* linn bark X 40

Physicochemical Characterizations

Size and shape of the particles

Sieve and microscopic methods were used to determine particle size and shape. 91% of the dried extract of *Mangifera indica* L.. passed through the *355 µm* (Sieve no. 44)and only 9 % passed through the 180 µm (sieve no. 80).

Solubility Test

Solubility's of the powdered extract were determined in solvents: ether, chloroform, ethanol, acetone, water and Ethanol:Water respectively. Excess amount of sample was added in 20 ml of solvent with stirring (300 rpm), at temperature 25 ± 0.5°C for 48 h and sonicated using sonicator for 2 hr. Samples were filtered through 0.45 µm filters and assayed spectrophotometrically for drug content at 316nm (Table 1).

Table 1: Solubility of the extract with different solvent

Parameters	% Solubility W/V
Solubility in ether	5 ± 2
Solubility of chloroform	36 ± 2.8
Solubility in ethanol	72 ± 6.3
Solubility in acetone	56 ± 4.3
Solubility in water	80 ± 1.8
Solubility in Ethanol:Water (80:20)	90 ± 3.6

Ash Values and Loss on drying

Determination of ash values such as total ash, acid insoluble ash, sulphated ash, and loss on drying is meant for detecting low grade products, exhausted drugs and sandy or earthy matters. Determination of ash values and loss on drying were carried out. The results are reported in Table no 2.

Table 2: Ash values of powder extract

Sr. No.	Ash Values	%W/W
1	Total Ash	2.775%
2	Acid insoluble	0.50%
3	Sulphated ash	3.54%
4	Loss on drying	4.8%

pH

pH was determined by shaking 1g of powdered extract in a 100 ml of freshly prepared and cooled distilled water in a vol. flask for 5 minute and the pH determined after filtration using a digital pH meter (Systronic Lab. Model 335) (Table 3).

Angle of repose

The static angle of repose (θ), was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel.The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation: tan θ = 2h/D

Bulk and Tap Densities

10g quantities of the powder sample (Passed through # 40)was placed in a 100 mL measuring cylinder and the volume, V_0, occupied by each of the samples without tapping was noted. After 10 taps on the table, in a constant distance the occupied volume V_{10} was read. The bulk and tap densities were calculated as the ratio of weight to volume (V_0 and V_{10} respectively).

Hausner's index

This was calculated as the ratio of tapped density to bulk density of the samples.

Hausner ratio = Dt/Db

Compressibility index (C %): This was calculated using the equation: C = Dt-Db/Dt*100

The obtained results are reported below in Table no 3.

Table 3: pH and Physical properties of the powder

Parameters	*Mangiferaindica* L. Extract
pH	6.2
Bulk density (g/cc)	0.74
Tapped density (g/cc)	0.92
Compressibility index	19.56%
Hausners quotient	1.24
Angle of repose	26.75º

Qualitative Phytochemical Screening

Extracts obtained from *Mangifera indica* L. bark were examined for the presence of various phytoconstituents by performing qualitative phytochemical tests and the results are recorded below in the table no 4.

Thin Layer Chromatography (TLC)

Dried mixture extract of *Mangifera indica* L. 5g was dissolved in 25ml of alcohol:water mixture (80:20) and filtered. This preparation was spotted in the TLC plate as test and for Standard 2g of mangiferin RS(reference standard) dissolved in alcohol:water (80:20) mixture.

Thermogravimetric analysis

The TGA was used to determine the weight loss of the material on heating. Transitions involving mass changes are detected by TGA as a function of temperature and time. The TGA curve for the extract are as shown below in Fig no:4 showed that extract did not decompose before 400^0c. The extract underwent 14.59% weight loss at 200^0C.It implies that the extract obtained from *Mangifera indica* L. Bark has excellent thermal stability (Fig 4).

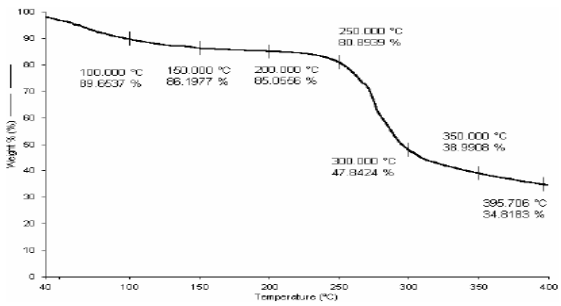

Figure 4: Thermogravimetric analysis of *Mangifera indica* L.

Microstructure Studies

The Extract samples for the purpose of microstructure analysis were subjected to x2000 magnification. The result showed in Fig no.5 indicates that extract is composed of thread shaped oblong crystal (Fig.5).

Table 4: Qualitative Phytochemical Screening

S/N	Tests	RS	S/N	Tests	RS	S/N	Tests	RS
1	**Alkaloids**		4	**Carbohydrates**		7	**Tannins**	
a	Dragendorff's test	-	a	Fehling's test	+	a	Ferric chloride test	+
b	Hager's test	-	b	Molisch's test	+	b	Lead acetate test	+
c	Wagner's test	-	c	Benedict's test	+	8	**Phenolic's**	
d	Mayer's test	-	d	Barfoed's test	+	a	Gelatin test	+
2	**Steroids**		5	**Protein's**	+	b	Aqueous bromine test	+
a	Salkowski test	+	a	Biuret's test		9	**Glycoside's**	
b	Liebermann Burchard's test	+	b	Xanthoproteic's Test	-	a	Killer killani test	+
3	**Flavonoids**		c	Million's test	-	b	Legal's test	+
a	Shinoda's test	+	d	Ninhydrin's test	-	c	Borntrager's test	+
b	Lead acetate test	+	6	**Saponin's**				
			a	Foam test	+			

RS: Results, - : Absent, **+ :** Present

Table 5: Thin layer chromatography (TLC) of the bark extract of Mangifera indica L.

No of Spot		Solvent system	Spraying Reagent	Rf Value	Remark
Test Standard (Mangiferin)	1 1	Ethyl acetate: Formic acid:acetic acid :Water(100:11:11:25)	Vanillin-Glacial acetic acid	0.4483 0.4413	Indicates the presence of Xanthone glycoside. It confirmsthe presence of Mangiferin.
Test Standard	2(Trailing) 1	Ethyl acetate: Formic acid:acetic acid :Water(100:11:11:25)	Ferric chlo-ride reagent	0.396 &0.434 0.393	Indicates the presence of Phenolic compounds and Tannins
Test Standard	2 Nil	Ethyl acetate: Formic acid:acetic acid :Water(100:11:11:25)	Libermann Burchard reagent	0.25, 0.30 -	Indicates the presence of Terpins and Steroids.

Table 6: Results of Total aerobic bacterial count and absence of *E. coli, Salmonella, Shigella and C. albicans*

	No. of CFU per plate after incubation				
	TAC	**E. coli**	**Salmonella**	**Shigella**	**C.albicans**
+ Control	200	40	35	25	10
- Control	NIL	NIL	NIL	NIL	NIL
Sample	15	NIL	NIL	NIL	NIL

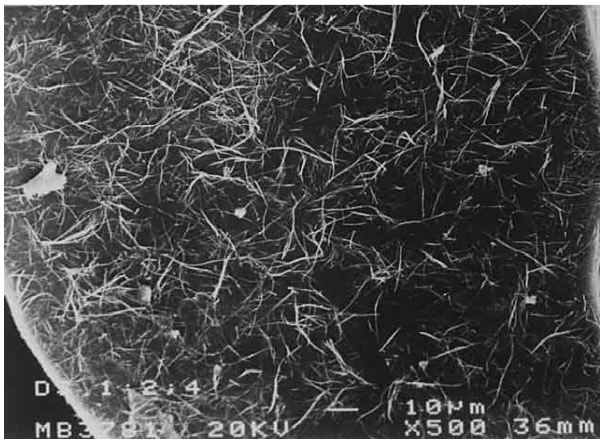

Figure 5: x 2000 magnification

Microbiological studies

The result of microbiological observations are given in the table 7.

CONCLUSION

Indian system of medicine uses majority of crude drug extract that are of plant origin. It is absolutely necessary that the standards are laid down to control and check the identity of the plant and their extract to ascertain the quality before it is used. A detailed pharmacognostical, physicochemical and phytochemical studies are essential to start work on formulation.

The present investigation has developed specific anatomical characteristics of *M. indica* L. bark in addition to its physicochemical attributes which are helpful for the specification development and standardisation of the plant bark. Also the present study revealed that as a part of preformulation study, the dried hydroalcoholic extract of the bark showed promising physicochemical characteristics. This will certainly help in the further formulation development work which will increase usefulness of the plant in medicine. In the pre-

sent investigation a detailed preformulation studies have been carried out which will help to develop formulation containing *Mangifera indica* L. Patient compliance and use of *Mangifera indica* L. extract will increase, once suitable dosage forms are developed.

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