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Pharmacognosy and quality control analysis of flower of *Madhuca indica* J. F. Gmel

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Abstract
Ayurveda herbs are in great demand these days. *Madhuca indica* commonly known as *Mahua*, belonging to the family Sapotaceae is a medium sized deciduous tree which occurs in mixed deciduous forests throughout India. All the parts of plant *viz*. Root, Bark, Stem, Leaves, Flowers, Fruits, Seeds, Oil possess medicinal properties. Flowers are being used in diarrhea and dysentery, coughs, colds, bronchitis, wasting disorders and as food. Flowers contain cane sugar, cellulose, albuminous substances, protein, phosphorous, calcium, iron, magnesium and copper which may be responsible for its various actions. The plant has huge economic potential due to various medicinal and other uses but is not being utilized fully due to lack of awareness & proper research. The present work embodies the investigations carried out to establish methods for identification of the purity and quality control of flower of the plant as per WHO guidelines. Complete phyto- pharmacognostical evaluation which comprises macroscopic, microscopic, phytochemical evaluation and physicochemical parameters like loss on drying, extractive value, ash value has been done with the aim to provide referential information for the correct identification & standardisation of the crude drug and hence will help to select the correct sample.

Keywords: *Madhuca indica*, flowers, pharmacognostical, identification, standardisation

Introduction

Madhuca indica J.F.Gmel. Syn. *M. latifolia* (Roxb.) Macbride, *Bassia latifolia* Roxb. (Fam. Sapotaceae) [1]; is found in the state Dehradun, Saharanpur, Chota Nagpur, Siwaliks, Uttar Pradesh, Madhya Pradesh, Orissa, Chhattisgarh, Jharkhand, Gujarat, Andhra Pradesh, Maharashtra, Bihar, West Bengal, North circars, Deccan and Karnataka [2].

The tree grows on a wide variety of soils but thrives best on sandy soil. It also grows on shallow, bouldery, clayey and calcareous soils. It is found up to an altitude of 1200m, mean annual maximum temperature 28-50°C, minimum 2-12°C; annual rainfall from 550-1500 mm. The species is drought-resistant, strong light demander and readily suppressed under shade. It is not frost-hard [3].

Flowers are small and fleshy, yellow in color and in define fascicles near end of branches. Corolla tubular, freshly pale, yellow aromatic and caduceus. Fruits are 2-6 cm long, fleshy and greenish. The bark is thick, dark colored, cracked, inner bark dark red, trunk short, branches numerous. Leaves are 10-30 cm long, thick and leathery, most of leaves pointed at the tip, clustescent glabrred near end of branches, epileptic or elliptic oblong [4].

Mahua is one of those multipurpose forest tree species that provide an answer for the three major Fs *i.e.* food, fodder and fuel. It is greatly valued for its flowers and its seeds known as tora. The tree has religious and aesthetic value in the tribal culture [5]. *Mahua* flowers are well known for their high reducing sugar and nutrient content. They are edible and used as a sweetener in preparation of many local dishes like *halwa*, *kheer*, *puri* and *burfi* in the *mahua* production belt of India [6]. Every part of any plant possess some medicinal properties, either in small or large proportion. The plant consist of several parts *viz*. root, bark, leaves, flowers, fruits, seeds, oil which may be classified according to the function [7].

Madhuca indica flowers are largely used in India, in diarrhea and dysentery, and as food [8]. They are used in coughs, colds, bronchitis and wasting disorders [9]. The fresh juice is alterative and given in scrofula and rheumatic affections [10]. Dried flowers used as a fomentation in orchitis [11]. The honey from the flower is edible and is reported to be used for eye diseases [12]. The flowers are useful in impotence [13]. They are also eaten by people suffering from piles after frying in *ghee* [14]. An infusion of the flowers is given with sugar, for the relief of thirst, burning of the body, giddiness [15], cough and fever [16].

Polysaccharide, PS-AI, isolated from flowers; hydrolysis gave galactose, arabinose, rhamnose, xylose and glucoronic acid in molar ratio of 21:5:1:1:6 respectively (*Carbohydr. Res. 1983, 112,113; Chem. Abstr. 1983, 98, 14376 n; Carbohydr. Res. 1984, 125145; Chem. Abstr. 1984, 100, 82776 c*); polysaccharide, PS-AII, also isolated from flowers, was constituted of galactose, glucose, arabinose and glucoronic acid (*Carbohydr. Res.1984, 127,283; Chem. Abstr. 1984, 101, 3911 n*). Flowers contain cane sugar, cellulose, albuminous substances and ash [17]. The corollas are also a rich source of phosphorous, calcium and iron; magnesium and copper are also present [18]. Protein quality of flower is superior and comparable with groundnut protein (*Jayasree et al. 1998*).

As per *Ayurveda*, *Madhuca indica* has *Madhura-Kashaya Rasa*; *Madhura Vipaka*; *Guru-Snigdha Guna* & *Sheeta Veerya.* Owing to its sweetness it is also known as *Gudapushpa*, *Madhupushpa*, *Madhusrava* [20]. Various formulations are prepared from it *viz*. *Madhookasava*,

Drakshadi kwatha churna, *Eladi Modaka* and is therapeutically used for *Trishna*, *Daha*, *Shrama*, *Swasa*, *Kshata*, *Kshaya* [19].

Material and Method

Plant Material: The flowers of *Madhuca indica* were collected from District Sonbhadra, U.P. and authenticated by NISCAIR, New Delhi, vide no:- NISCAIR/RHMD/Consult/2018/3173-22. Collected plant material was washed thoroughly with water and dried under shade. Dried flowers were powdered in a grinder and the powder was extracted with different solvents such as Water & Ethanol by cold maceration method and Petroleum ether by soxhlation process. The extracts were evaporated to dryness at a controlled temperature (65ºC).

Microscopic study: Transverse section of flower of *Madhuca indica* J.F. Gmel was taken, stained with safranine and observed under the microscope and then cellular structure was identified.

Determination of Foreign matter [21]: Weigh 100-500 g of the drug sample to be examined and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens. Separate and weigh it and calculate the percentage present.

Determination of pH [22]: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidic or basic nature of a solution.

- The pH of given solution was measured by using digital pH meter.
- Firstly, the pH meter was standardized. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH.
- The instrument was switched on and Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water.
- The sample was taken (10% W/V aqueous solution) and electrode was dipped in it and the value of pH was noted.

Determination of Moisture Content [23]: Moisture content was determined by placing weighed sample of 2 to 5gm of drug in oven at 105º C for 5 hours, and calculating weight of sample for every 30 minute, until the weight of the sample came out to be constant i.e. no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing. And calculate percentage moisture content.

Determination of Alcohol Soluble Extractive Value[22]: 5 g coarsely powdered air dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105º, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Determination of Water Soluble Extractive Value: Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

Determination of Petroleum Ether Extractive Value: Take air dried & crushed 20-25 gm sample (W_1) in extraction thimble. Extract with 150 ml solvent ether (Petroleum Ether, B. P. 40^0 to 60^0 C.) in a continuous extraction apparatus (Soxhlet Apparatus) for approx. 6 hours, until extractor and thistle tube having colourless solvent. Take & weigh empty Petri dish/ Flat bottom shallow dish (W_2). Filter the extract quantitatively into a Tared Evaporating dish/ Petri dish. Evaporate the solvent on a water bath. Dry the residue at 105^0 C upto constant weight. Take weight of Petri dish with Extract/ Residue (W_3). Calculate the percentage of ether soluble extractive with reference to the air-dried drug.

Determination of Total Ash [24]: Silica Crucible was cleaned, dried well, labelled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample was put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 600°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

Determination of Acid Insoluble Ash: Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

Determination of Water-soluble Ash: Water – soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch‘s Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

Phytochemical Analysis

Table 1: Procedure of Phytochemical Tests

Name of Test	Procedure	Observation	Result
Carbohydrates			
Molisch's test	2 ml Test Solution + 2 ml Molisch's reagent & shake carefully + 1ml. of conc. H_2SO_4 Wait for one 1 minute.	A Purple colour ring at the junction of the two layers	Carbohydrate present
Benedict's test	4 ml Test solution + 1 ml Benedict's solution + ▲	Formation of green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.	Reducing sugars present
Fehling's test	Fehling A 1 ml + Fehling B 1 ml + 2 ml Test solution + ▲	Brick Red ppt.	Generally indicates presence of reducing sugars
Barfoed's test	Test sample + Barfoed's reagent + ▲	Red precipitate of cuprous oxide within two minutes	Presence of monosaccharides.
Alkaloids			
Dragendorff test	2 ml test Solution + 2 ml Dragendorff reagent	Orange precipitate	Alkaloids
Wagner's test	Test solution + few drops of Wagner's reagent	reddish-brown precipitate	
Hager's test	Test solution + Hager's reagent	Orange yellow precipitate	Presence of alkaloids.
Amino acids			
Ninhydrin	Test solution + Ninhydrin + ▲	characteristic deep blue or pale yellow colour	Presence of alpha-amino acids and proteins containing free amino groups.
Protein			
Biuret test	Test solution + 1 ml of 4% NaOH solution + 1 drop of 1% solution of $CuSo_4$.	Development of violet or pink colour	Presence of proteins.
Xanthoprotic test	Test sample + 2 ml of water + 0.5 ml of conc. HNO_3	Development of yellow colour	Presence of proteins.
Millon's test	Test solution + 2-3 ml of Millons reagent was added.	White precipitate slowly turning to pink	Presence of proteins.
Saponin			
Foam test	Test solution + sodium bicarbonate + Water.	A stable, characteristic honeycomb like froth	Presence of saponins.
Glycosides			
Borntrager's test	1 ml Benzene + 0.5 ml Dil. NH_4 Sol. + Test Solution	Formation of reddish pink colour.	
Phenolic compound			
Phenolic test	Test Solution + ▲ + 2 ml of $FeCl_3$ sol.	Formation of green and blue colour.	
Steroids			
Salkowski	Test Solution + 2 ml of chloroform + 2 ml of conc. H_2SO_4 & shake for few minutes	Development of red colour	Presence of steroids.
Tannins			
$Fecl_3$	Test Solution + 5 % solution of $FeCl_3$ in 90 % alcohol	Appearance of dark green or deep blue colour	Presence of tannins.
Lead acetate	Test Solution + 10 percent w/v solution of basic lead acetate in distilled water	Development of precipitate	Presence of tannins.
Pot. Dichromate	Test Solution + Potassium dichromate Solution	Appearance of dark colour	Presence of tannins.

Chromatography [25]

Plate preparation: Precoated T.L.C. plate with 0.25 mm Layer of silica gel G60F 254 with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of plate: Plates were dried in hot oven at 105^0 C for one and half hour.

Preparation of mobile solution: Toluene: Ethyl Acetate (7:3)

Test sample: Ethanol extract of flower and concentrated juices (prepared by Direct and Indirect heat)

Sample application: Sample was applied with the help of capillary 1 cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 cm below the top of the T.L.C. plate.

Visualization with spraying reagents: *p-Anisaldehyde – sulphuric acid*

Rf Value: Measured and recorded the distance of each spot from the point of its application and calculated Rf value by dividing the distance travelled by the spots (Solute/Sample) by the distance travelled by the mobile phase (Mixture of different Solvents).

$$\text{Rf Value} = \frac{\text{Distance travelled by the spots (Solute/Sample)}}{\text{Distance travelled by the mobile phase (Mixture of different Solvents)}}$$

Distance travelled by the mobile phase (Mixture of different Solvents)

Observation and Result

Macroscopy

Fresh Flower: Stalked and drooping flowers with Fleshy, tubular corolla

Dry Flower: Like dried raisins

Fig 1: Fresh and dried Flowers of *Madhuca indica* J. F. Gmel

Fig 2: Opened view of part of fleshy Corolla

Table 2: Results of Organoleptic examination

Examination	Fresh Flower	Dry Flower
Taste	Sweet and juicy	Sweet
Odor	Sweet	Sweet
Touch	Soft	Rough
Colour	Yellow	Reddish brown

Corolla: Fleshy, tubular, lobes 8- 10, gamopetalous, free at top, Flat and hollow at base enclosing 20- 25 epipetalous stamens (Fig 2)

Anther: Sub- sessile, basifixed, lanceolate, pointed at tip and hairy at the back with prominent dark brown connective strand (Fig 2)

Microscopy

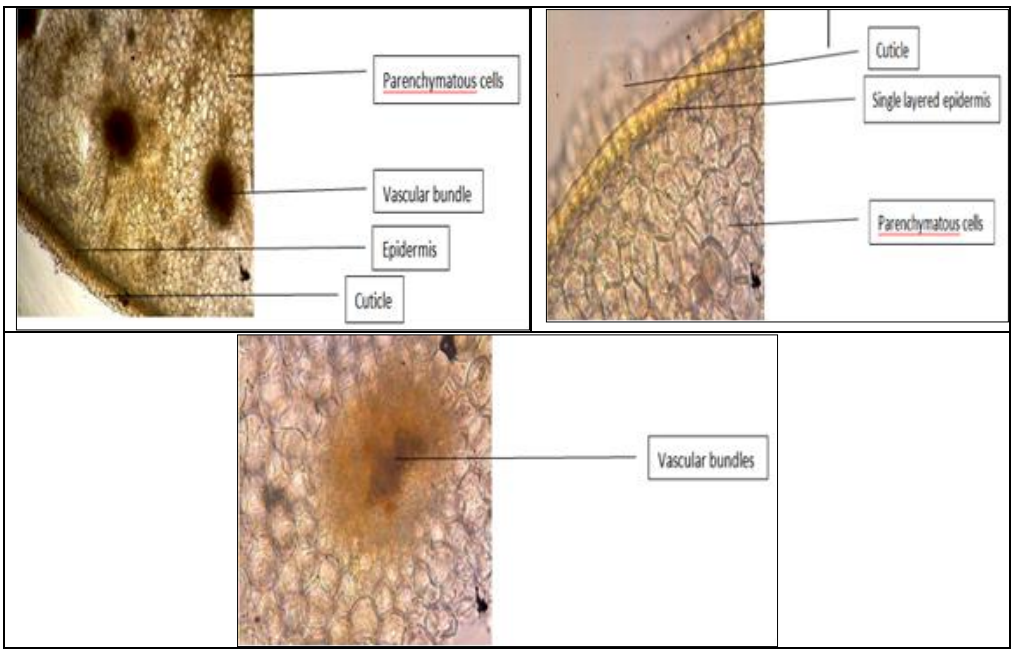

Fig 3: TS of Petal of *Madhuca indica* flower

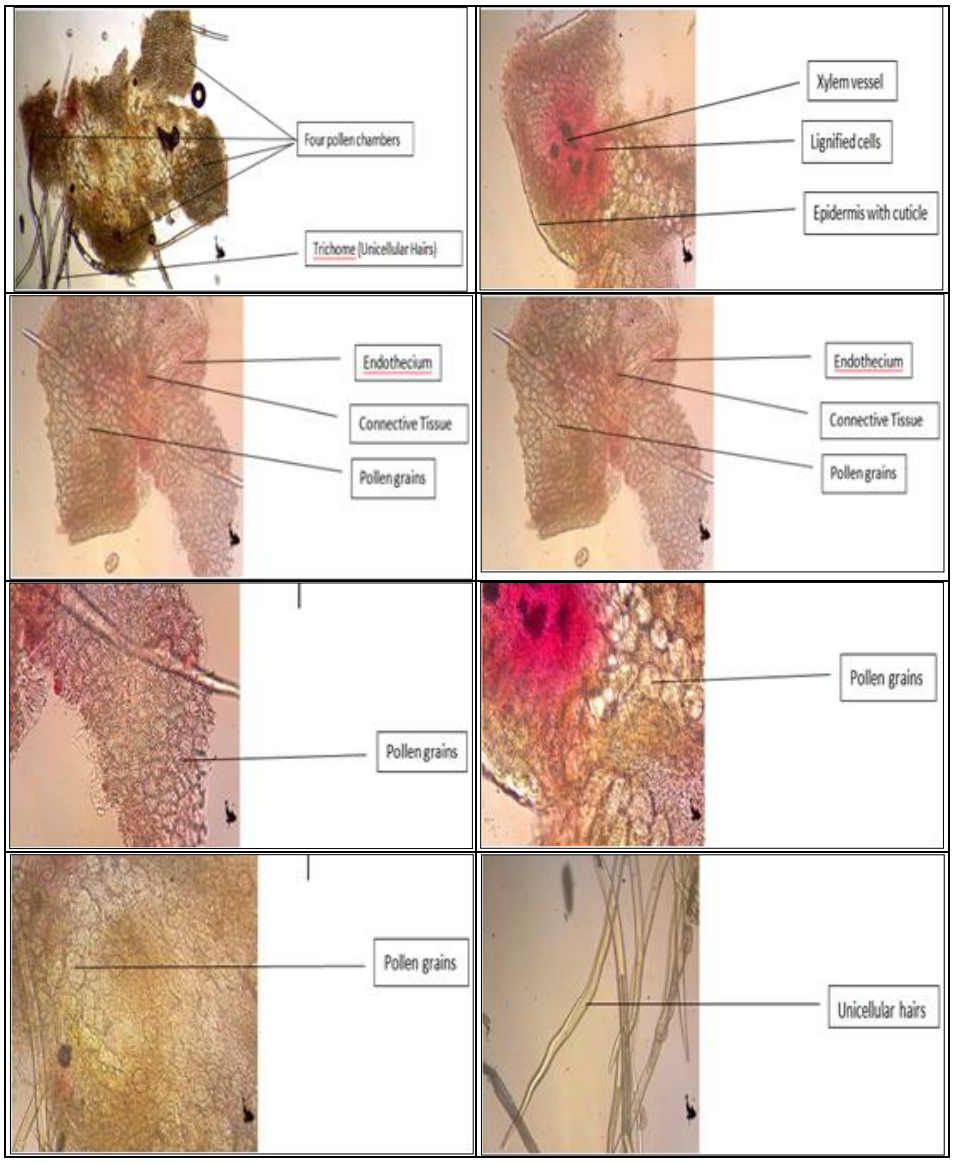

Fig 4: TS of Anther of *Madhuca indica* flower

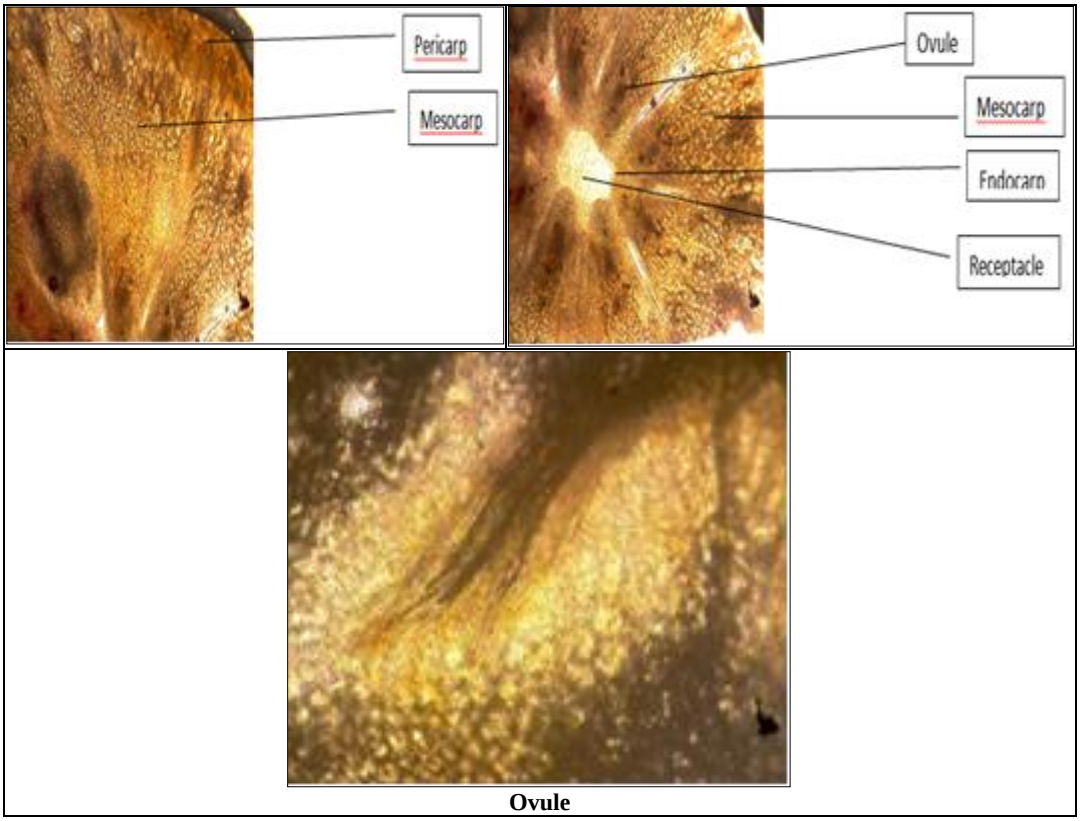

Fig 5: TS of Ovary of *Madhuca indica* flower

T.S of petal showed a single layered epidermis, followed by thin-walled, irregularly shaped parenchymatous cells, vascular bundles found scattered in parenchymatous tissues (Fig 3).

TS of Anther showed 4 pollen chambers and prominent cells of connective tissue in the centre of the chambers, epidermis single layered covered with thin cuticle, unicellular hairs are present, endothecium composed of radially elongated, oval shaped, lignified cells. Pollen grains are present in groups and having spherical in shape (Fig 4). Pericarp, endocarp, mesocarp, receptacle and ovule were identified in TS of ovary (Fig 5).

Physiochemical Parameters

Table 3: Results of Physicochemical Tests

S. No.	Test	Result	API Limit
1.	Foreign matter	1.23%	NMT 2%
2.	Moisture Content	8.38%	NMT 10%
3.	pH	5.1	---
4.	Total Ash	4.32%	NMT 5%
5.	Acid Insoluble Ash	0.21%	NMT 0.5%
6.	Water Soluble Ash	3.67%	---
7.	Aqueous Extractive Value	72.34%	NLT 70%
8	Alcoholic Extractive Value	23.58%	NLT 25%
9.	Ether Extractive Value	2.74%	---

Table 4: Phytochemical Profile of Flower *Madhuca indica*.

Name of Test	Aqueous Extract	Alcoholic Extract	Petroleum ether Extract
Carbohydrate			
Molisch's test	+ve	-ve	-ve
Benedict's test	-ve	-ve	-ve
Fehling's test	+ve	+ve	-ve
Barfoed's test	-ve	-ve	-ve
Alkaloids			
Dragendorff test	+ve	-ve	-ve
Wagner's test	-ve	-ve	-ve
Hager's test	+ve	+ve	-ve
Amino acids			
Ninhydrin	+ve	-ve	+ve
Protein			
Biuret test	+ve	+ve	-ve
Xanthoprotic test	+ve	+ve	+ve
Millon's test	+ve	+ve	-ve
Saponin			
Foam test	+ve	-ve	-ve
Glycosides			
Borntrager's test	+ve	-ve	-ve
Phenolic compound			
Phenolic test	+ve	+ve	+ve

Steroids			
Salkowski	+ve	-ve	-ve
Tannins			
$Fecl_3$	+ve	+ve	+ve
Lead acetate	+ve	+ve	-ve
Pot. Dichromate	-ve	+ve	-ve

Table 5: Thin Layer Chromatography Profile of alcoholic extract of Flower of *Madhuca indica*.

Mobile Solution: Toluene: Ethyl Acetate (7:3)
Stationary Phase: Precoated Silica Gel G Plate
Visualization with *p-Anisaldehyde – sulphuric acid*
R_f Value:- 0.09, 0.23, 0.44, 0.49, 0.61, 0.65, 0.67, 0.72, 0.84

Discussion

Fresh flowers were yellow in color, soft in touch, sweet in odor, sweet and juicy in taste whereas dried flowers were Reddish brown in color, rough in touch, sweet in odor and taste. Specific macroscopic characters *viz*. corolla fleshy, tubular with 8- 10 lobes, gamopetalous, free at top, flat & hollow at base enclosing 20- 25 epipetalous stamens; and specific microscopic characters *viz*. vascular bundles scattered in parenchymatous tissues of petals; anthers showing 4 pollen chambers, prominent cells of connective tissue in the center and unicellular trichomes; proved to be useful tool for identification of *Madhuca indica* flower.

Flower has acidic (pH 5.1) nature. Foreign matter was 1.23%. Moisture content of powdered flower was found to be 8.38% w/w. Total ash which represents total inorganic material present in flower powder was 4.32% w/w; acid insoluble ash (i.e. Silicaceous material) being 0.21% and carbonates, sulphates & other organic compounds being 3.67%. Aqueous extractive value i.e. water soluble phytochemicals present in flower were 72.34% w/w *viz*. Carbohydrate, Amino Acid, Saponin, Phenolic compound, Tannins; Alcoholic extractive value i.e. ethanol soluble phytochemicals present were 23.58% w/w i.e. Carbohydrate, Alkaloid, Glycoside, Tannins; Fat soluble content was 2.74% w/w.

Molisch's test, Fehling's test, Dragendorff test, Hager's test, Ninhydrin, Biuret test, Xanthoprotic test, Millon's test, Foam test, Borntrager's test, Phenolic test, Salkowski, $Fecl_3$, Lead acetate tests were positive in the aqueous extract which showed the presence of Carbohydrates, alkaloids, Amino acids, proteins, Saponins, glycosides, Phenolic compounds, steroids & Tannins. Fehling's test, Hager's test, Biuret test, Xanthoprotic test, Millon's test, Phenolic test, $Fecl_3$, Lead acetate, Pot. Dichromate tests were positive in the alcoholic extract which indicated the presence of Carbohydrate, Alkaloids, Protein, Phenolic compound and Tannins. Ninhydrin, Xanthoprotic test, Phenolic test and $Fecl_3$ test were positive in petroleum ether extract which indicated the presence of Amino acids, Protein, Phenolic compound and Tannins.

Phenols, sugars, steroids and terpenes had separated in Toluene: Ethyl Acetate (7:3) mobile solution and visualized under p-Anisaldehyde – sulphuric acid reagent and these chemical constituents had an identity feature in form of Rf value 0.09, 0.23, 0.44, 0.49, 0.65, 0.67, 0.72, 0.84 in flower.

Conclusion

Quality Control Parameters complied with reference value of API which revalidated them again.

The flowers contained most of the Primary (Carbohydrates, Amino acids, Proteins) and Secondary metabolites (Alkaloids, Saponin, Glycosides, Phenolic compound, Steroids and Tannin) which justified its various biological effects *viz*. nutritonal effects, antioxidant, hepatoprotective, etc. Nutritional efficacy was further re-established through nutritional analysis. Hence, pharmacognostical information of flower of *Madhuca indica* provided valuable information about its correct identity and evaluation. It will help to differentiate from the closely related other species of Madhuca. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The other parameters observed are also useful for the future identification of the plant and serves as a standard monograph for identification and evaluation of the plant.

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