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A Preliminary HPTLC fingerprint study of *Madhuka Indica* –An Immune Enhancer

Research Article

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Abstract

Ayurveda is now accepted as a global medical science. The world is shifting to herbal remedies which is considered to be safe and effective. But the quality maintenance of these herbal medicines is a difficult task which hinders the globalization of Ayurvedic drugs and it is a need to use suitable techniques to maintain relevant standards. Standardization is very much important for the reproducibility of the efficacy and safety of any drug which is brought to the counter as medicine for sale. *Madhuka* (1) is been mentioned in classical texts as *brimhana, veerya pushtivardhana, dahahara, shramahara, vatapittavinasham*, *bhutadi jantudoshagna, kshatkshayharam etc*. This article aims to explore the qualities of *Madhuka pushpa,* its HPTLC phytoconstituent fingerprint and the preliminary standardization of preparing *madhuka pushpa vati* for its use as an immune enhancer.

Key Words: *Madhuka puspha,* Immune enhancer, *Veerya pushtivardhana*, HPTLC

Introduction

Madhuka indica is a plant of Indian origin having tremendous therapeutic potential to provide health to the society, but it is not fully utilized and recognized. *Madhuka* commonly known as mahua or botanically named as *madhuka indica* from the sapotaceae family is a tropical tree found largely in the central and north plains and forests. *Madhuka* is used to prepare liquor and for fermentation process to make *asava* and *arishtas*. *Madhuka pushpa* has been indicated as *brimhana, veerya pushtivardhana.* These qualities itself indicates that *madhuka* has the capacity to improve all the seven *dhatus* or promoting the essence of all the seven *dhatus* (tissues) ie bringing about the *Prashasta Bhava* which may be equalent as an immune enhancer. Any drug may be of plant, animal or mineral origin may have varying properties as per their place of availability or climatic conditions. And hence in this present study *Madhuka pushpa vati* which was been prepared at a GMP certified pharmacy was subjected to standardization to maintain proper quality control, safety and efficacy of the medicine.

Aims and Objectives

1. Identification and authentification of raw drugs.
2. Preparation of *Madhuka pushpa vati*.
3. Phytochemical analysis and chromatography (HPTLC) of *Madhuka pushpa vati*.

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Materials and Methods

Collection of raw drugs

The raw drugs were procured from Udupi, Karnataka. The part used are given in the table No 1. The raw drug was identified as *Madhuka indica* belonging from Sapotaceae family which was certified from SDM Centre for Research in Ayurveda and Allied sciences, Udupi, Karnataka.

Table No. 1

Sr. No.	Name of Drug	Latin Name	Family	Part used
1	*Madhuka*	*Madhuka indica*	Sapotaceae	Flowers

Vernacular names (2,3,4)

- Sanskrit : Madhuka
- Classical name : Madhuka
- English : Indian butter tree
- Hindi : Mahua

Taxonomical position -

- Kingdom : Plantae
- Division : Magnoliophyta
- Subdivision : Angiosperm
- Class : Magnoliopsida
- Subclass : Caesalpinieace
- Order : Ericaleae
- Family : Sapotaceae
- Genus : Madhuka
- Species : indica, longifolia

Guna	***Madhuka***
Rasa	*Madhura Kashaya*
Veerya	*Sheeta*
Vipaka	*Madhura*
Guna	*Snigdha , guru*
Dosha	*Vata pitta hara*
Karma	*Brimhanam, Shukrakruta, Veerya pushti vivardhanam*

Methods of evaluation

Microscopy of *Madhuka pushpa* and HPTLC of *Madhuka pushpa vati* was analyzed by using standard qualitative and quantitative parameters. All the procedures were conducted at Sri Dharmasthala Manjunatheswara Centre for Research in Ayurveda and Allied sciences (AYUSH centre for Excellence and Recognized SIROs by DSIR)

Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features of *Madhuka indica* were compared to local flora for authentication.

Figure 1: Macroscopy of *Madhuka indica* flower

***Madhuka indica* flower**

Microscopy Study

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Description of flower

Macroscopic characters of flowers from Fig. 1

Drug consists of mostly corolla and androecium; corolla fleshy, reddish –brown, tubular, lobes 7-14(usually 8-9), ovate lanceolate, short, erect 0.5-2cm long; Stamensepipetalous, basifixed, lanceolate, pointed at tip and hairy at the back with prominent dark brown connective strand; Taste-sweet. Figure 2: Microscopy of *Madhuka Indica* flower.

Fig 2a. Microscopy of Corolla | **Fig 2b. Portion of corolla enlarged** | **Fig 2c. Mesophyll region**

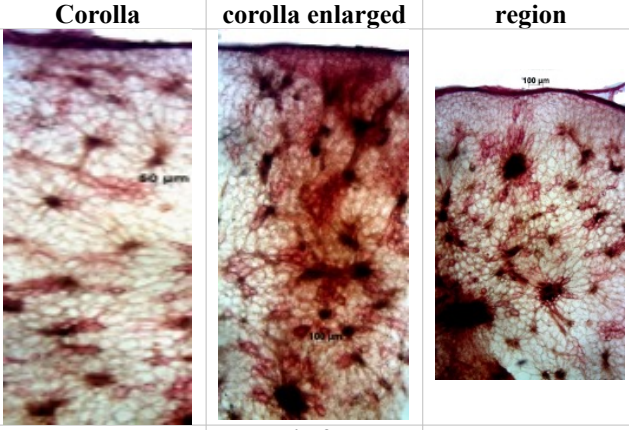
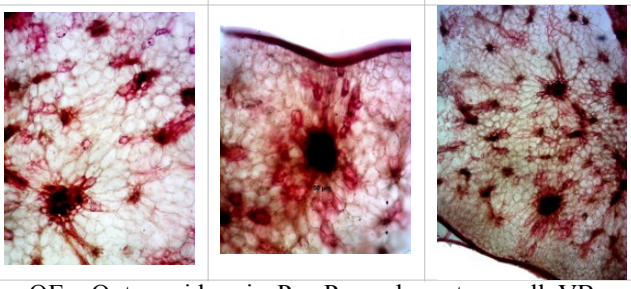


Fig 2d. Vascular bundle | **Fig 2e. Outer epidermis and vascular bundle** | **Fig 2f. Inner epidermis**



OE – Outer epidermis, Pa –Parenchymatous cell, VB- Vascular Bundle, IE- Inner epidermis

Microscopy of Madhuka flower from Fig. 2a -2f:

Corolla

Petal shows a single layered epidermis, followed by thin-walled, irregularly shaped parenchymatous cells; vascular bundles found scattered in parenchymatous tissues.

Androecium

Anther shows 4 pollen chambers and prominent cells of connective tissue in the centre of the chambers; epidermis single layered covered with thin cuticle; a few unicellular hairs present on one side; endothecium composed of radially elongated, oval shaped lignified cells; tapetum not distinct; pollen grains single or in groups, spherical, with clear exine and entine walls scattered in the pollen sac, a few cells of the vascular bundles are seen embedded in the connective tissues.

Standardisation of *Madhuka pushpa vati*

- **Preparation of *Madhuka pushpa vati:*** It was prepared in S.D.M College of Ayurveda pharmacy , Udupi.
- Method: *Madhuka pushpa* initially it was tray dried for 3-4 days. Later it was kept in dryer for 7-8 days with 48-50 degree Celsius under 760 mm of Hg pressure and later it was made into *vati* without using any binding agent. Each *vati* in the batch measured nearly 3grams with a hardness test of 1.0 kg/cm and disintegration time of 39 minutes. The prepared *vati* was solid with a shiny black appearance, sweet astringent in taste with a mild aroma. The results are depicted in table no 2 & 3 and the final product after making of vati is depicted in Figure no 3.

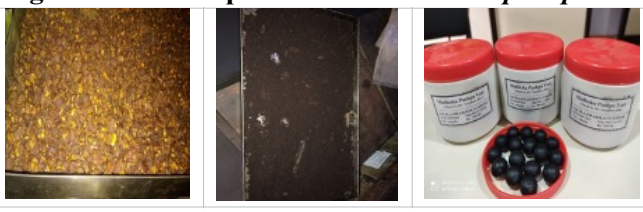
Table no 2. – Results of Standardization of *Madhuka pushpa vati*

Parameters	*Madhuka pushpa vati*
Tablet average wt	3.087
Tablet average wt (Average wt±SEM)	3.087±0.16
Variation in weight (%)	0.16
Hardness test (kg/cm)	1.0
Disintegration time (min)	39

Table no 3. - Organoleptic characteristics of *Madhuka pushpa vati*

Character	Observation
Color	Black
Odour	Aromatic
Taste	Sweet, Astringent
Consistency	Solid

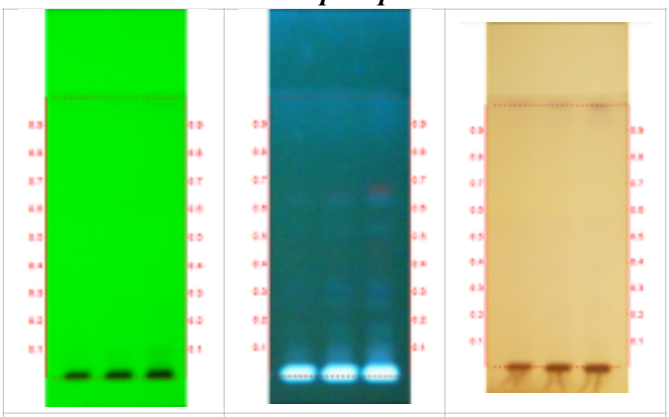
Figure No. 3 - Preparation of *Madhuka pushpa vati*



HPTLC study

Madhuka pushpa vati was subjected to HPTLC study at Sri Dharmasthala Manjunatheswara Centre for Research in Ayurveda and Allied sciences (AYUSH centre for Excellence and Recognized SIROs by DSIR) and the results with photo documentation after derivatisation is depicted in Figure 4 using linomat 5 applicator, CAMAG visualization chamber and CAMAG scanner 4 with wincats software.

Figure 4: HPTLC Photodocumentation of sample of *Madhukapushpa Vati*



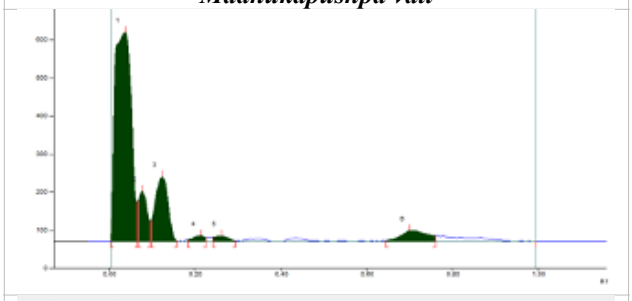
Short UV	Long UV	After derivatisation

Track 1: ***Madhukapushpa vati***- 3µl
Track 2: ***Madhukapushpa vati***- 6µl
Track 3: ***Madhukapushpa vati***- 9µl
Solvent system- Toluene: Ethyl acetate (7.0: 1.0)

Table 4: Rf values of sample *Madhuka Pushpa vati*

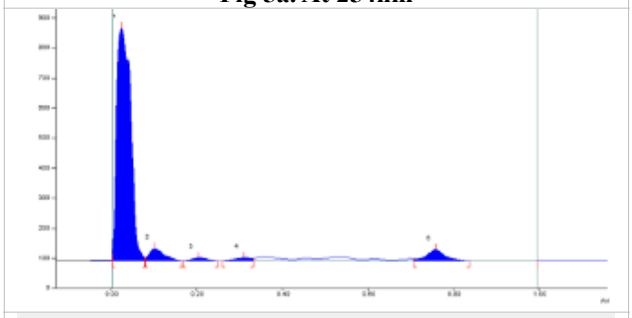
Short UV	Long UV	After derivatisation
-	0.08 (F. blue)	-
-	0.19 (F. blue)	-
-	0.27 (F. blue)	-
-	0.34 (F. blue)	-
-	0.53 (F. blue)	-
-	0.63 (F. blue)	-
-	0.67 (F. red)	-

Figure 5: Densitometric scan of the sample of *Madhukapushpa vati*



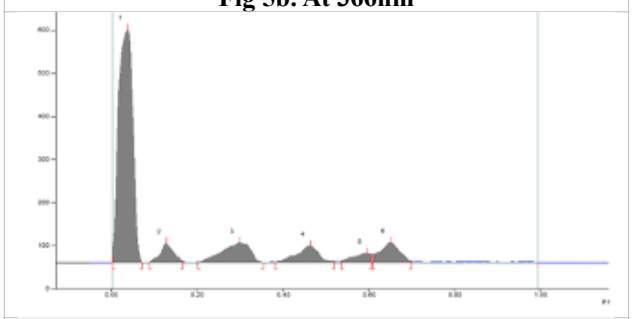
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.04 Rf	549.0 AU	60.41 %	0.07 Rf	93.8 AU	15219.0 AU	67.09 %
2	0.07 Rf	106.1 AU	0.09 Rf	131.9 AU	14.52 %	0.10 Rf	52.8 AU	1923.0 AU	8.48 %
3	0.10 Rf	55.0 AU	0.12 Rf	169.9 AU	18.69 %	0.16 Rf	1.3 AU	3668.6 AU	16.17 %
4	0.18 Rf	3.7 AU	0.21 Rf	15.8 AU	1.86 %	0.23 Rf	9.9 AU	287.2 AU	1.27 %
5	0.24 Rf	10.6 AU	0.26 Rf	14.1 AU	1.56 %	0.30 Rf	1.4 AU	327.1 AU	1.44 %
6	0.65 Rf	2.1 AU	0.70 Rf	28.8 AU	3.17 %	0.76 Rf	14.5 AU	1260.3 AU	5.56 %

Fig 5a. At 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	19.8 AU	0.02 Rf	774.5 AU	88.79 %	0.08 Rf	11.2 AU	18442.3 AU	87.19 %
2	0.08 Rf	11.3 AU	0.10 Rf	40.6 AU	4.65 %	0.17 Rf	0.4 AU	1020.1 AU	4.82 %
3	0.17 Rf	0.5 AU	0.20 Rf	10.1 AU	1.18 %	0.25 Rf	0.0 AU	254.4 AU	1.20 %
4	0.26 Rf	0.0 AU	0.31 Rf	10.3 AU	1.18 %	0.33 Rf	5.3 AU	288.6 AU	1.36 %
5	0.71 Rf	5.4 AU	0.76 Rf	36.8 AU	4.22 %	0.84 Rf	0.1 AU	1148.4 AU	5.42 %

Fig 5b. At 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	16.1 AU	0.04 Rf	538.7 AU	73.18 %	0.07 Rf	0.4 AU	12992.5 AU	85.12 %
2	0.09 Rf	0.4 AU	0.13 Rf	44.3 AU	6.02 %	0.17 Rf	3.9 AU	1016.2 AU	5.09 %
3	0.20 Rf	1.2 AU	0.30 Rf	46.0 AU	6.25 %	0.36 Rf	0.4 AU	2237.5 AU	11.21 %
4	0.38 Rf	0.2 AU	0.47 Rf	38.9 AU	5.28 %	0.52 Rf	3.5 AU	1479.6 AU	7.42 %
5	0.54 Rf	4.8 AU	0.60 Rf	21.6 AU	2.94 %	0.61 Rf	19.8 AU	675.0 AU	3.38 %
6	0.81 Rf	19.1 AU	0.85 Rf	48.7 AU	6.34 %	0.70 Rf	4.2 AU	1551.6 AU	7.78 %

Fig 5c. At 620nm

Results on HPTLC of *Madhuka pushpa vati*

HPTLC plate was applied with ethanolic extract of sample at 3, 6, 9µl with linomat 5 applicator, was developed in Toluene: Ethyl acetate (7:1) solvent system and the developed plates following drying were observed in CAMAG visualization chamber followed by scanning at 254nm,366nm,620nm using CAMAG scanner 4. After derivatization, plate was examined for appearance of different bands at different R_f and following were the findings

1. *Madhukapushpa vati* at Short UV shows no bands; at long UV it shows 7 bands with R_f at 0.08, 0.19, 0.27, 0.34, 0.53, 0.63, 0.67, after derivatization with ASA (anisaldehyde sulphuric acid) reagent there were no bands prominently evident.
2. At 254nm desnsitometric scan shows 6 peaks at R_f 0.04(67.09%), 0.08 (8.48%), 0.12 (16.17%), 0.21 (1.27%), 0.26 (1.44%), 0.70 (5.56%) among which notable one was 0.12(16.17%). At 366nm densitometric scan shows 5 peaks at R_f 0.02, 0.10, 0.20, 0.31, 0.76 among which major peaks were at 0.10(4.82%), 0.76(5.42%). At 620nm (Following derivatisation with Anisaldehyde sulphuric acid reagent) 6 peaks were depicted among which 0.30(11.21%), 0.65(7.78%) were prominent.

Thus, *Madhuka pushpa vati* was found to be rich in phytoconstituents.

Conclusion

Madhuka indica with *madhura rasa, snigdha guru guna* and *sheeta veerya madhura vipaka* is indicated as *brimhana , shukrakara* and *vatapitta shamaka.* Hence it is said to improve the body tissues, specifically *shukra dhatu as the panchabhautika guna* of *madhuka pushpa and shukra is similar* and therefore improving the *ojus* or immunity. *Madhuka pushpa vati* were analysed with organoleptic characters, phytochemical parameters and chromatographic study. Standard quality techniques of *Vati kalpana* were adopted in making of *Madhuka pushpa vati*. In future for further exploration of *Madhuka indica* this study will helpful to prepare a monograph for Ayurvedic Formulary of India (AFI).

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