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PHYTOCHEMICAL AND PHARMACOGNOSTICAL EVALUATION OF MADANPHALA PIPPALI (*Catunaregam spinosa* Thunb) COLLECTED FROM IT'S HABITAT AND CRUDE DRUG MARKET

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Abstract: *Dravya (food and drug substances) plays a vital role in the treatment of disease.The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. In those days, the physician himself identifies, checks the drugs based on habitat, morphology, taste, colour, texture and uses as medicine. Madanaphala is considered as the best amongst the Vamana Dravya. In Charaka Samhita, Madanaphala is ascribed as the best drug for Vamana. It's other applications as Asthapana basti, Anuvasana basti and udara lepa has also been described.*

For the best results of Madanaphala, standardization of drug is very necessary. The quality of the drug can be assessed on the basis of the chromatographic fingerprint (TLC). Madanaphala were collected from two places. First from its natural habitat (Forestry region of Mahabaleshwar, Satara district of Maharashtra state) and processed it according to classcis and second was obtained from the crude drug market (Goladinanath, Varanasi, Uttar pradesh). After phytochemical and pharmacognostical evaluation it was found that the sample obtained from it's habitat is better as compare to the market sample.

Keywords: *Madanaphala, Pippali, Catunaregam spinosa Thunb, Vamana, Asthapan basti, Anuvasan, TLC.*

Introduction: Panchakarma is one of the procedures to eliminate the doshas, with the help of plant based drugs. The Panchakarma comprises of; Vamana, Virechana, Anuvasana, Asthapana and Shirovirechana. The foremost one is well established procedure to eliminates the doshas particularly Kapha from oro-gastric route, which is commonly known as Vamana [1]. Madanaphala is considered as the best amongst the Vamana Dravya [2]. It's other applications as Asthapana basti, Anuvasana basti [3] and udara lepa [4] has also been described in ayurvedic classics.

The specific collection time as well as procedure [5] to obtain standard quality of drug has been followed in our study. World Health Organization's publication has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards.Under these circumstances, pharmacognosy, pharmacology and phytochemistry are necessary for authentification of crude drug and to prove therapeutic action as well.

Materials and Methods

Madanaphala were collected from 2 places. First from its natural habitat (Forestry region of Mahabaleshwar, Satara district of Maharashtra state) and processed it according to classcis and second was obtained from the crude drug market (Goladinanath, Varanasi, Uttar pradesh). Macroscopic and microscopic evaluation was carried out with seed. Seed was pulverized in the mechanical grinder to a moderate fine powder to carry out microscopic studies and was stored in a well closed airtight vessel for further analysis. All reagent and chemicals used for the study were of analytical grade.

Pharmacognostical Study

Macroscopic and Microscopic Description of Madanaphala: Madana consists of dried fruit of *Catunaregam spinosa* (Thunb), (Fam. Rubiaceae), a deciduous thorny shrub, reaching a height up to 9 meter and girth about a meter,

branches numerous, thick and horizontal, found in sub-Himalayan tracts extending eastwards in Sikkim up to 1200 m and southwards to peninsular India [6].

a) Macroscopic Study

Fruit: 1.8-4.5 cm long, globose or broadly ovoid, longitudinally ribbed or smooth yellowish-brown, crowned with persistent calyx-limb, fruit, contains numerous seeds, 0.4-0.6 cm long, compressed, smooth, brown and very hard [7].

b) Microscopic Study

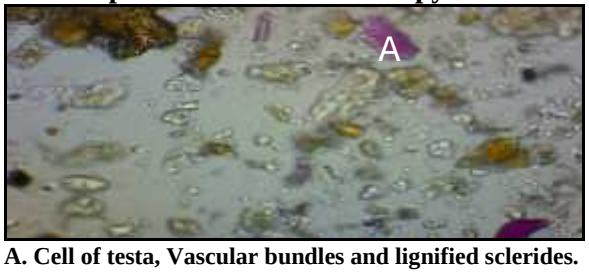
Fruit: Transverse section shows epicarp consisting of single layered epidermis, sometimes obliterated in surface view, epidermal cells thin-walled and polygonal, mesocarp, broad zone consisting of thin-walled, parenchyamatous cells, some cells contain reddish - brown content, a number of vascular bundles found embedded in this zone, endocarp stony consisting of light yellow polygonal, sclerenchymatous cells of variable shape and size.

Seed: Transverse section shows a fruit pulp, consisting of single layered, rounded to oval epidermal cells, a few layers of yellowish-brown pigmented cells, and endosperm forms bulk of seed consisting of large oval and irregular shaped parenchymatous cells, albumen horny, translucent, cells of outermost layer smaller in size.

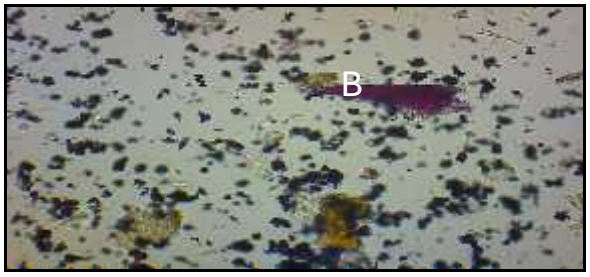
Powder: Reddish brown, under microscope shows numerous, large, irregular, reddish brown cells sclereids of variable shape and size, pieces of xylem vessels with reticulate thickenings, thin- walled, crushed parenchymatous cells and yellow - orange pieces of fruit pulp [8].

Fruit powder shows tannin containing cells of mesocarp as such or overlapping with the polygonal cells of epicarp in surface view, tracheids and fibres of the vascular bundles, group of stone cells of the endocarp associated with fibres; testa in surface view; endosperm with aleurone grains and oil globules [9].

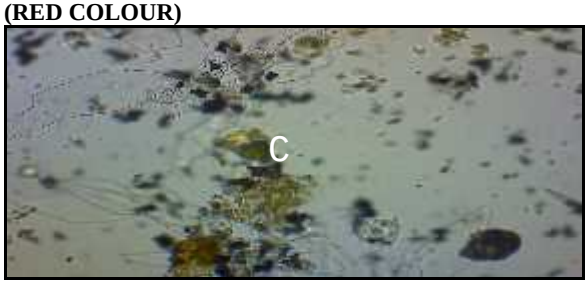
Madanaphala: Powder Microscopy



A. Cell of testa, Vascular bundles and lignified sclerides. (RED COLOUR)



B. Oil Globules (Mesocarp and endosperm), Cuticle. (RED COLOUR)



C. Aleurone grain (Endosperm) (Yellow Colour)

Determination of Foreign Matter (WHO 2002): 100 g of the powdered sample of the crude drug was weighed accurately. It was then spread in a thin layer on a white tile uniformly without overlapping and the inspection was done with naked eyes or with the help of a 6X lens. The foreign matter was then separated manually as completely as possible. The portions of this sorted foreign matter were weighed and the content of each group in grams per 100g of air-dried sample was calculated.

Determination of Loss on Drying (WHO 2002): A glass-stoppered, shallow weighing bottle was weighed, accurately 2 g of the specified sample was weighed and then transferred to the bottle and the bottle containing the content was again accurately weighed. The sample was then distributed as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm. The loaded bottles were placed in the drying chamber (hot air oven). Eventually the sample was then dried to constant weight for the specified time and at the temperature (over 110˚C). After the drying was completed the bottle was promptly removed and allowed to cool at room temperature in desiccators before weighing. Finally the weight of the bottle along with the contents was weighed and the percentage loss in weight was calculated.

Determination of Ash Value (WHO 2002)

Total Ash (WHO 2002): Accurately 2 g of the ground air-dried material was weighed and placed in a previously ignited and tarred silica crucible. The powder sample was then spread evenly and then was incinerated to a constant weight by gradually increasing the temperature to 500-600˚C until it was white, indicating the

absence of carbon. The crucible was then cooled in a desiccator and finally weighed. The content of total ash in terms of percentage w/w of air dried material was calculated.

Acid Insoluble Ash (WHO 2002): The crucible containing the total ash obtained after incineration was further boiled gently for 5 minutes with 25 ml of hydrochloric acid (~70 g/litre) and was then covered with a watch glass. The watch glass was then rinsed with 5 ml of hot water and this liquid was then added to the crucible. The insoluble matter was then collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was then transferred to the crucible, dried on a hot-plate and then was ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The content of acid-insoluble ash was calculated in mg per g of air-dried material.

Water Soluble Ash (WHO 2002): The crucible containing total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was then collected on an ash less filter paper. It was then washed with hot water and finally ignited in a crucible for 15 minutes at a temperature not exceeding 450˚C. The percentage of water soluble ash in mg per g of air-dried material was reported.

Physiochemical Parameters

Parameters	**Results**	
	***Madanaphala* (Natural habitat)**	***Madanaphala* (Market sample)**
Percentage of Foreign Matter	Nil	Nil
Loss on Drying	3.8% w/w	4.3% w/w
Total Ash	7.7% w/w	6.2% w/w
Acid Insoluble Ash	3.6% w/w	2.6% w/w
Water Soluble Ash	4.5% w/w	5.5% w/w
Swelling Index	3.2 ml/g	3.2 ml/g
Foaming Index	100	100

All the reading were calculated in triplicate.

Extractive Value: 4.0 g of coarsely powdered air-dried material was accurately weighed and placed in a glass-stoppered conical flask. Powder was then macerated with 100 ml of the solvent (Water/ethanol) concerned for 6 hours, shaking frequently, and then was allowed to stand for 18 hours. It was then filtered rapidly taking care not to lose any solvent; 25 ml of this filtrate was transferred to a tarred flat-bottomed dish and was evaporated to dryness on a water-bath. It was followed by drying at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was weighed without delay. The content of extractable matter in mg per g of air-dried material was then calculated.

Extractive Value Findings

Extract	**Report % w/w**	
	***Madanaphala* (Natural habitat)**	***Madanaphala* (Market sample)**
Water	10.2	10.2
Ethanol	15.4	15.4

All the reading were calculated in triplicate.

Thin Layer Chromatography: Thin Layer Chromatography (TLC) is an extremely useful technique for preliminary identification and separation of phytoconstituents. The optimum separation of compounds by TLC is usually achieved when R_f values are between 0.15–0.85.

R_f = Distance from origin to center of spot/Distance from origin to solvent front

Procedure: Stationary Phase: The stationary phase used was TLC Silica gel 60 F_{254} aluminum sheets.

Preparation of the Sample Extract: The sample plant extract was prepared simply by just dissolving the required quantity of the extract in ethanol.

Solvent system	**Madanaphala pippali (Natural habitat)**			**Madanaphala fruit pulp (Natural habitat)**			**Madanaphala pippali (Market sample)**		
	A	B	R_f	A	b	R_f	A	B	R_f
Toluene: methanol: water (7:2:1)	3 cm	4.5 cm	0.66	2.9 cm	5 cm	0.58	3.5 cm	5 cm	0.7
Toluene: ethanol: water (6:2:2)	2 cm	4.5 cm	0.44	3 cm	5 cm	0.6	3.2 cm	5 cm	0.64

a= Distance travelled by the solute, b= Distance travelled by the solvent

R_f value= a/b = Distance travelled by the solute/ Distance travelled by the solvent

Natural habitat (Phala Pippali)

Natural habitat (Fruit pulp)

Market sample (Phala Pippali)

Developed TLC plates and seen in UV 366 nm Solvent system; Toluene: methanol: water (7:2:1)

Phytochemical Study

Aim: To perform the phytochemical screening for the following extract sample:

1. Madanphala pippali (Natural habitat)
2. Madanphala Fruit pulp (Natural habitat)
3. Madanphala pippali (Market sample)

50% hydro alcoholic extracts were obtained by hot extraction (soxhlet method) and they were subjected to preliminary phytochemical screening for the presence of various phytoconstituents using various qualitative reagents [10].

S. No.	Phytoconstituents	Test	Madanaphala pippali (Natural habitat)	Madanaphala pippali (Market sample)	Madanaphala fruit pulp (Natural habitat)
1.	Amino acids	Test for Cysteine	+	+	+
2.	Alkaloids	Dragendorff's test and Mayer's test	–	–	+
3.	Flavonoids	Shinoda test and Alkaline reagent test	+	+	+
4.	Proteins	Biuret test and Xanthoproteic test	+	+	+
5.	Steroids	Salkowski test	+	+	_
6.	Phenols	5% $FeCl_3$ solution:	+	+	_
7.	Tannins	Dilute iodine solution	+	+	_
8.	Glycosides	Borntrager's test	+	+	+
9.	Saponins	Foam test	+	+	_

Discussion and Conclusion

It is evident that the proper collection time and there after adoption of proper procedure as laid down in Caraka Samhita from it's habitat as compare to market drug has scientifically proved that the former is best than the later one. Though , the other procedure was also adopted in market drug sample but its collection time as well as duration of storage is unknown to the drug traders, might be the factor for the inferior efficacy. In crude drug market, the dried fruit of following botanical source has been reported and sometimes sold as substitutes for the fruits of *Catunaregam spinosa;* they are *Gardenia turgida* Roxb. (Rubiaceae) and *Artabotrys hexapetalus* Linn. (Rubiaceae). Retail market price of dry fruits of *Madanaphala* is Rs- 40/-per kg. and is sold by the trade name; *Mainphala*.

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