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Quality Control Studies of *Mesua ferrea* Linn. Flowers

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Mesua ferrea Linn. belonging to the family Clusiaceae (alternatively Guttiferea). The flowers were fragrant, cream coloured, ebracteate, pedicellate, pedicel short, axillary, solitary or in pairs (cluster) and 2.5-7.5 cm in diameter, bisexual and buds were sub-globose. The transverse section showed numerous multicellular and multiseriate type trichomes on the upper layer of the epidermis. Foreign organic matter (0.47% w/w), total ash (6.30% w/w), acid insoluble ash (2.30 % w/w), water soluble ash (2.31 % w/w), alcohol soluble extractive (20.53 % w/w), water soluble extractive (10.26 % w/w), loss on drying (9.88 %), foaming index (333.3), swelling index (0.53 ml) and volatile oil (0.20 % v/w) of the crude drug were been obtained. A fingerprint of fluorescence was obtained. Chlorinated pesticide in first and second elute from column were 0.030 and 0.013 respectively and phosphated pesticides in first elute was 0.028 mg/kg of crude drug. Preliminary phytochemical screening showed the presence of many phytochemicals.

Keyword: *Mesua ferrea*, Fluorescence Analysis, Preliminary Phytochemical Screening Quantitative Standards

1. Introduction

Mesua ferrea Linn. belonging to the family Clusiaceae (alternatively Guttiferea). In Hindi it is known as'Nagakesara' and in English as Ceylon Ironwood. It is a medium to large evergreen tree with short trunk, often buttressed at the base, found in the Himalayas from Nepal eastward, in north-eastern India, Deccan Peninsula and the Andaman Islands, ascending to an altitude of 1.500m. The tree is cultivated in the gardens and avenues for its flowers and foliage which are attractive, particularly when young. Its flowers and leaves are used as an antidote to snake bite, and a paste of the flowers with butter and sugar is used in bleeding piles and burning of the feet. The oil of the seeds is used as an embrocation in rheumatism and found useful in the treatment of itch[1]. The plant is also used as antimicrobial[2,3,4,5,6], antiprotozoal and antibacterials on some gram positive bacteria[6], cytotoxic toward T-lymphocyte leukemia cells and weak antimicrobial[7], CNS depressant, anti-inflammatory and anti-ulcers[8]. The plant contains glycosides, coumarins, flavanoids, xanthones, triglycerides and resins. Essential oils, fatty acids, steroids, reducing sugar, tannin, saponin, proteins. The flower comtains α - copaene and germacrene D[9], stamens gave β-amyrin, β-sitosterol, and a new cyclohexadione compound named as mesuaferrol (I)[11] and mesuanic acid[11], the hexane and benzene extracts showed the presence of triterpenoids and resins, while the alcoholic and H_2O extracts were rich in reducing sugars, tannins and saponins

respectively[12]. The present study was designed to develop the different salient characteristics of the flower of the plant for its pharmacognostical standardization and quality control.

2. Materials and Methods

2.1 Collection and Authentication of the Plant Material

The plant *Mesua ferrea* Linn. produces blossoms in the month of January-March. The flowers were procured from Athagada, Odisha, India. The authentication as well as identification of Nagakesara was done by Prof. S. D. Dubey, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Science, Banaras Hindu University, Varanasi and a voucher specimen (COG/H.No. – 025) has been preserved for further references.

2.2 Morphological Studies

The morphological characters of the flower were studied according to the method of Brain and Turner[13,14,15].

2.3 Microscopical Studies

2.3.1 Preliminary Treatment

Dried flower were softened before they were cut for the purpose of preparation of microscopy, preferably by being placing them in a moist atmosphere, or by soaking them few minutes in boiling water.

2.3.2 Preparation of Specimens

Powdered material: Initially 1 to 2 drop of water, glycerol/ethanol or chloral hydrate was placed on a glass slide. Moistened the tip of the needle with water and dipped into the powdered drug, further a small quantity of this moist powder was then transferred into the glass slide containing the drop of the respective fluid. Stirred thoroughly but carefully and then applied a cover-glass over it. Press lightly on the cover-glass with the handle of the needle, and finally the excess fluid was removed from the margin of the cover-glass using a strip of filter paper.

Surface tissues of flowers: This is mostly performed on the sepals and petals of the flower depending on their texture and thickness. To render them transparent the cut portion was boiled carefully on a slide using chloral hydrate as the clearing agent over a small flame of a micro burner. This was done to remove bubbles and as soon as the bubbles have ceased the boiling was again continued until the fragments are transparent.

Sections: Mostly section were taken in cross (or transverse section) or longitudinal one. This was done by moistening the surface to be cut and the blade with ethanol (~375gm/litre). The section was then taken free handed as thinly and evenly as possible. Finally the section was transferred to a dish containing ethanol (~150gm/litre). The satisfactory good section was then selected, prepared and mounted on a slide which was finally ready for observation under the microscope.

2.4 Fluorescence Powder Drug Analysis

Fluorescence analysis was carried out according the method of Chase and Pratt (1949)[16].

2.5 Extraction and Preliminary Phytochemical Studies

The crude drugs were dried under shade for 4-6 days. It was then powdered in a mixer grinder until a suitable sized was achieved. Then it was then made to pass through sieve number 22/60. The coarse crude drug was then placed in a soxhlet extractor for the extraction purpose and 10% of the fines were added. The powder was then initially defatted with 40-60˚C petroleum ether for 15 hour in three days (5 hour per day) until the fatty material had extinguished, then was removed and air dried and finally followed with a 24 hours methanolic extraction for 4

days (6 hour per day). The methanolic extract was screened for the presence of various classes of phytoconstituents as per Khandelwal, K.R., 2005[17].

2.6 Determination of Quantitative Standacrds

Foreign matter, loss on drying, different ash values viz. total ash, acid insoluble ash and water soluble ash, alcohol soluble and water soluble extractive, foaming index and swelling index, chlorinated and phosphate pesticides and volatile oil were determined by following the methods from WHO guidelines, 1998 and Indian Herbal Pharmacoepeia, 2002[14,15].

3 Results and Discussions

3.1 Morphological Characters

The flowers (Fig. 1) were fragrant, cream coloured, ebracteate, pedicellate, pedicel short, axillary or terminal, solitary or in pairs (cluster) and 2.5-7.5 cm in diameter, bisexual**,** large, sub-sessile and buds were sub-globose, bracts nil. After the flower parts were dissected it was found that all the four whorl of the flower parts were seen to be clearly visible. Sepals were seen to be 4 in number, 2 outer slightly shorter than the inner ones and depressed at the based, orbicular, cubbed and puberulous. Petals were 4 in number, pure white fragrant, spreading, obovate-cuneate, with crisped and undulate margin often torn. Stamens were very numerous golden yellow united much shorter than the petals and were slightly united at the based into a fleshy ring. Filaments were small and anthers oblong. Ovary was seen to be superior, bicarpellary, syncarpous, style was found to be twice as long as the stamens, stigmas capitate, style and stigma persistent in young fruit but are shaded away later on.

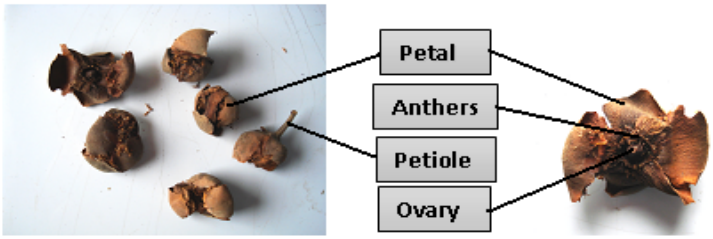

Fig 1: Crude drug of *Mesua ferrea* flower

3.2 Microscopical Characters

3.2.1 Transverse and Longitudinal Section Through Petals (Fig. 2 and Fig. 3)

The transverse section showed that the section composes of a two layered epidermis upper and lower one. Numerous trichomes were found to be present on the upper layer of the epidermis. Trichomes having the characteristics which are multicellular multiseriate type. The epidermis constitutes of a thick cuticle and was formed by few layers of cells. Below the epidermis was the ground tissue (cortex region) made of several layers of rounded collenchymatous cells. The cortex also constitutes of sclerenchymatous celled layers that was made up of sclerenchymatous fibres when viewed in longitudinal section. In addtition several numerous oil glands were also seen scattered in the cortical region. The ground tissue layer was followed by the lower epidermis which was also made up of few layers of round collenchymarous cells.

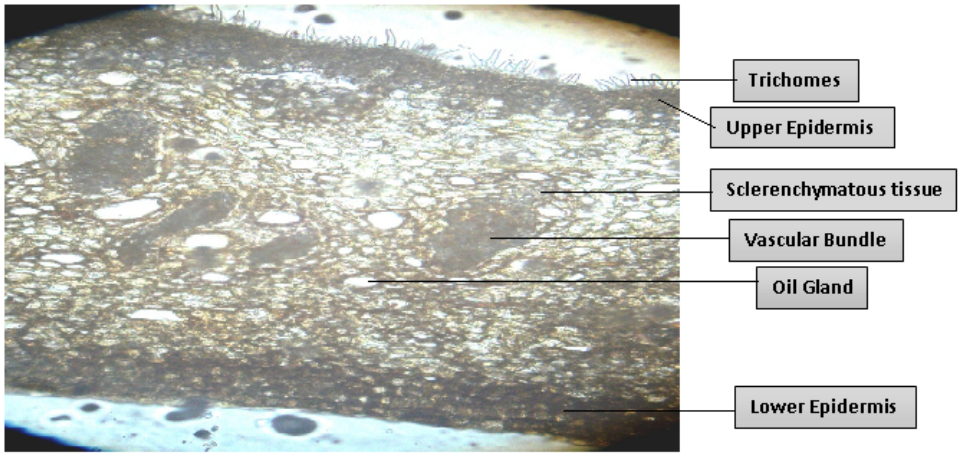

Fig. 2: Transverse section of *Mesua ferrea* crude drug through petal

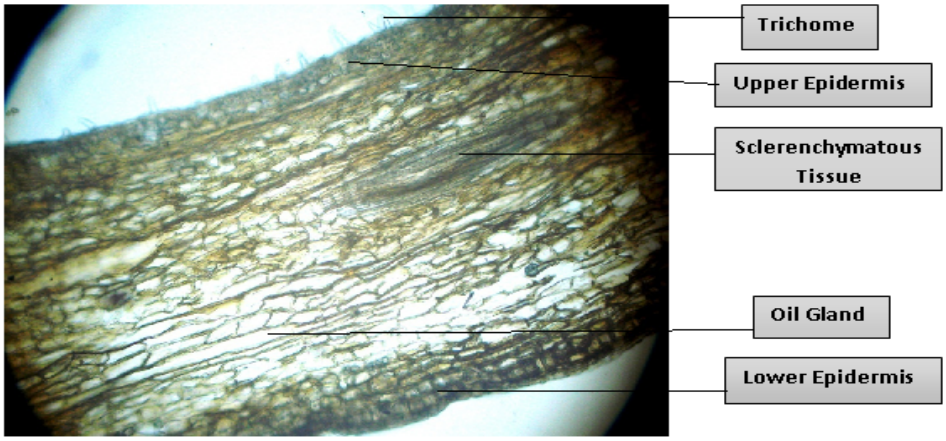

Fig. 3: Longitudinal section of *Mesua ferrea* crude drug through petals

3.2.2 Transverse Section Through the Ovary

The transverse section through the ovary shows the clear visbility of the ovary with its two ovules arranged in axile placentation manner. The clear figure of this section has been shown in the Fig. 4.

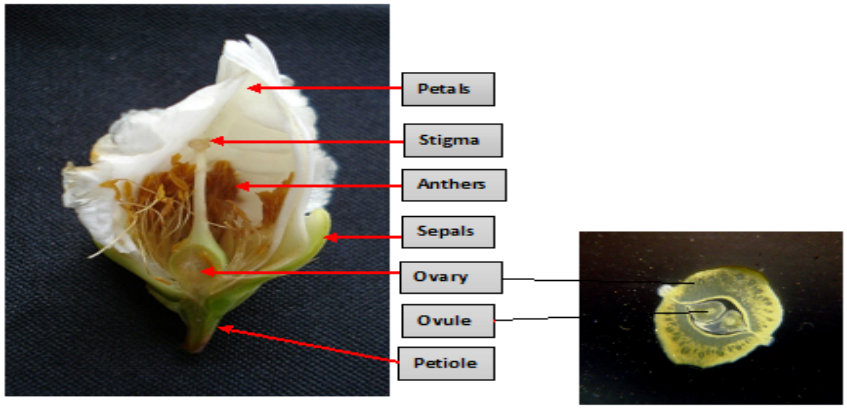

Fig. 4: Transverse section of *Mesua ferrea* fresh flower through ovary

3.3 Fluorescence Analysis

The results of fluorescence analysis of the drug powder have been presented in Table 1.

Table 1: Fluorescence powder drug analysis of *Mesua ferrea*

Sl.no	Powder + Reagent	Fluorescence in daylight	Fluorescence under UV (365nm)
1.	Powder as such	Brown (165,42,42) (A52A2A)	No Fluorescence (NF)
2.	Powder + 1N NaOH in methanol	Yellow 255,255,0 (FFFF00)	Green yellow (173,255,47) (ADFF2F)
3.	Powder + 1N NaoH in water	Dark red (139,0,0) (8B0000)	NF
4.	Powder + 1N HCl in methanol	Saddle Brown (139,69,19) (8B4513)	Light green (144,238,144) (90EE90)
5.	Powder + 1N HCl in water	Golden rod (218,165,32) (DAA520)	Pale green (152,251,152) (98FB98)
6.	Powder + 1N HNO_3 in methanol	Brown (165,42,42) (A52A2A)	Yellow green (154,205,50) (9ACD32)
7.	Powder + 1N HNO_3 in water	Light yellow (255,255,224) (FFFFE0)	Light green (144,238,144) (90EE90)
8.	Powder + Iodine (5%)	Dark golden rod (184,134,11) (B8860B)	Dark sea green (143,188,143) (8FBC8F)
9.	Powder + $FeCl_3$ (5%)	Dark olive green (85,107,47)	NF
10.	Powder + KOH (50%)	Brown (165,42,42) (A52A2A)	NF
11.	Powder + Ammonia (25%)	Dark golden rod (184,134,11) (B8860B)	Light green 9144,238,144) (90EE90)
12.	Powder + Picric acid (saturated)	Yellow (255,255,0) (FFFF00)	NF
13.	Powder + Acetic acid	Peru (205,133,63) (FFDAB9)	Light green (144,238,144) (90EE90)

Fluorescence analysis of the drug powder gives a fingerprint of colour pattern that helps in identification.

3.4 Preliminary Phytochemical Screening

The methanolic extract of the flower of the plant was found to contain alkaloids, glycosides, reducing sugar, tannins and

phenolics, coumarins, sterols, flavonoids, saponins and volatile oil as given in Table 2.

Table 2: Preliminary Phytochemical Screening of the Methanolic Extract

Phytoconstituent	Result
Alkaloids	++
Glycosides	+
Reducing sugar	++
Tannins & Phenolics	++
Coumarins	++
Sterols	+
Flavanoids	++
Saponins	++
Proteins	-
Mucilage	-
Volatile oil	++

(-): Absent
(+): Present in small quantity
(++): Present in good quantity (Intense colour / more precipitate)
The above result shows the presence of almost all classes of phytoconstituents.

3.5 Quantitative Standards

The various quantitative standards have been furnished in Table 3 and 4.

Table 3: Determination of quantitative standards

Quantitative standards	Value
Foreign matter	0.47 w/w %
Loss on drying	9.88 w/w %
Total ash	6.30 w/w %
Acid insoluble ash	2.30 w/w %
Water soluble ash	2.31 w/w %
Alcoholic Soluble Extractive	20.53 w/w %
Water Soluble Extractive	10.26 w/w %
Foaming Index	333.3
Swelling Index	0.53 ml
Volatile oil	0.20% v/w

Note: All the above values are average of three readings

The value of water soluble extractive indicates the presence of lesser amounts of carbohydrates and glycosides. High foaming index is the indication of either presence of high amount of saponins in the crude drug.

Table 4: Determination of Pesticide Residue of *Mesua ferrea*

Samples	Concentration of pesticides (in mg/kg of crude drug)	
	Chlorinated pesticide	Phosphate pesticide
TS1	0.030	0.028
TS2	0.013	NA
TS3	NA	NA

TS1: First elute containing Chlorinated pesticide
TS2: Second elute containing Chlorinated pesticide
TS1: First elute containing Phosphated pesticide
TS2: Second elute containing Phosphated pesticide
TS3: Third elute containing Phosphated pesticide
NA: Not applicable

Since both pesticide residues and heavy metal contents are negligible, hence the crude drug may be considered safe for biological use.

4 Conclusion

The various morphological, microscopical and quantitative standards developed in this study will help for botanical identification, quality control and standardization of the drug 164in crude form. Further, the authentic plant material can be explored for its 165pharmacological and phytochemical potential.

5 Acknowledgement

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