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### **Research Article**

# A PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDY ON THE MARKET SAMPLES OF *NAGAKESARA*

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### ABSTRACT

This work was conducted to evaluate the pharmacognostic and phytochemical study of varieties of *Nagakesara*. When it is further studied, it is observed that *Nagakesara* available in the markets of different areas is from different source plants. There are nearly 5 drugs sold in the market with the same name, the common floral parts available in the markets are commonly from *Nagakesara* (*Mesua ferrea* Linn.), *Surapunnaga* (*Ochrocarpus longifolius* Benth and Hook f.), *Tamalpatra* (*Cinnamomum tamala* Nees and Ebern.), *Punnaga* (*Calophyllum inophyllum* Linn.), *Dillenia pentagyna* Roxb. Hence a comparative study of these two samples 1) *Nagakesara* (*Mesua ferrea*), and 2) *Tamalpatra* (*Cinnamomum tamala*) has been carried out. The phytochemical study shows the presence of tannins, steroids and carbohydrates in almost all varieties of *Nagakesara*. Flower buds of *Nagakesara* plant of different species available in the market was taken up for the study. **CONCLUSION**: A detailed Pharamcognostic and Phytochemical review was done through which it was concluded that *Mesua ferrea* Linn. belonging to family Guttiferae may be the exact source of *Nagakesara*. The flower buds of *Cinnamomum tamala* Nees and Ebern. which is known as black variety in the markets according to our study.

**KEYWORDS:** Nagakesara, Mesua ferrea Linn., Surapunnaga, Ochrocarpus longifolius Benth and Hook f., Tamalpatra, Cinnamomum tamala Nees and Ebern, Punnaga, Calophyllum inophyllum Linn.

### INTRODUCTION

Nagakesara mentioned in classical works has become controversial because of improper identification, using the drug with different names in different areas and many other contributing factors like cheating trade. It is found that there is a lot of confusion regarding the acceptance of genuine drug under the name Nagakesara. When it is further studied, it is observed that Nagakesara available in the markets of different areas is from different source plants. There are nearly 5 drugs sold in the market with the same name, the common floral parts available in the markets are commonly from Nagakesara (Mesua ferrea Linn.), Surapunnaga (Ochrocarpus longifolius Benth and Hook f.), Tamalpatra (Cinnamomum tamala Nees and Ebern.), Punnaga (Calophyllum inophyllum Linn.), Dillenia pentagyna Roxb. The synonyms like Nagapuspam, Nagam, Kesaram, Campeyam (because of its creamy yellow colour), Nagakinjalakam, Kanakahvaya, Sthalaja, Ahipuspa, Kanchana, Phanikesara indicate the colour and shape of stigma and style and the female part of the flower look like serpents hood. These synonyms created a lot of confusion in Nagakesara varieties, as the Nagapuspa and Kesara

are using synonymously for almost all species. Due to less availability of Mesua ferrea Linn., some other species are used as Nagakesara. Nearest point of availability is one of the major reasons for Substitution. Laal Nagakesara: immature floral buds of Surapunnaga (Ochrocarpus longifolius Benth and Hook f.) are sold in Gujarat, Maharashtra and Hyderabad Punnaga markets. (Calophyllum inophyllum Linn.) flowers are used as substitute for Nagakesara due to resemblance of size, shape and colour. Malabaar Nagakesara: The unripe fruits of *Cinnamomum wightii* or *Cinnamomum tamala* Nees and Ebern are sold in the markets of Chennai, which is considered as a substitute and adulterant for *Nagakesara*, which are black in colour, when dried.

Hence a comparative study of these two samples 1) *Nagakesara* (*Mesua ferrea*), and 2) *Tamalpatra* (*Cinnamomum tamala*) has been carried out in present study. The information provided by this study provides relevant pharmacognostical and physico chemical data which was needed for proper identification and authentication of *Nagakesara* varieties.

### **Review of Literature**

Comparative morphology of various samples of *Nagakesara* 

Morphology:

### Mesua Ferrea:[1] Guttiferae

**Habitat:** Commonly found on the Eastern Himalayas, Eastern Bengal and Assam, Eastern and Western Ghats which grows upto 5000feet and cultivated in gardens. **Habit:** Medium sized tree with short trunk. **Leaves:** Young leaves are red in colour, lanceolate, coriaceous, covered with a waxy bloom underneath, red when young. **Petioles:** 6-8 mm. long. **Flowers:** Ovoid with long persistent calyx.; buds are subglobose. **Stamens:** Numerous with golden-yellow colour, much shorter than the petals.





Tree



Flower



**Showing Useful Parts** 



**Young Leaves** 



Stamens







Seeds

**Fruit:** 2. 5-3 cm. long, ovoid with a conical point. **Seeds:** 1 to 4, dark brown, upto with fleshy oil.

### Morphology of dried flowers of Mesua Ferrea

Calyx and corolla are in goldish brown colour, coppery and gold filaments are seen on anthers, with persistent petiole and ovary. Numerous stamens are seen measuring approximately 0.5–1.5cms and having astringent taste.

## Ochrocarpus longifolius benth. & hook. F<sup>[2]</sup>

### Family: Guttiferae

**Habitat:** Available in Western Ghats of the Konkan, North Kanara, Malabar and Coimbatore. **Habit** - A large tree and young shoots are terete. **Leaves** oblong, obtuse, coriaceous, bluntly pointed, base rounded, petioles are long, stout. **Flowers-** numerous in numbers which are globose, apiculate and orangered in colour; Calyx dividing into 2 valves, reflexed during flowering. Petals are acute and 4 in number, ovate-oblong, acute, thin, deciduous, young buds are red in colour. Stamens many, sterile in the female flowers. **Fruit-** 2.5cm, long, ovoid, orange red in colour and seeded.

### Morphology of Dried Flowers Buds of Ochrocarpus Longifolius

Buds are black to brownish in colour and round in shape, Presence of persistent odour even after drying of flower bud and having astringent taste.

### Cinnamomum Tamala Nees and Ebern<sup>[3]</sup>

### Family: Lauraceae

**Habitat**: Found in Tropical and subtropical Himalaya, grows to a height of 3000-7800 feet and in Sylhet and Khasia Hilla, grows to a height of 3000-4000ft. **Habit**: A small evergreen tree grows upto 1.4 m. **Bark**: Dark brown or black in colour, slightly rough in nature, pinkish or reddish-brown in colour with whitish



**Dried Flowers** 

streaks present in the exterior. **Leaves:** Opposite, alternate, ovate, oblong or lanceolate glabrous, shining above and red in colour, **Flower**: pale yellow in colour arranged in axillary and terminal panicles filaments villous. **Fruit**: Drupe, ovoid, fleshy, black.



Dried Flower Buds of Tamalpatra (Cinnamomum Tamala)

# Morphology of dried flowers buds of *Cinnamomum Tamala*

Dried buds are globose to round in shape with dark brown colour and having small persistent calyx with strong odour.

### Calophyllum Inophyllum Linn<sup>[4]</sup>

**Habitat**: Available in Eastern and Western coasts of the Peninsula, Burma, the Andamans and Malay Peninsula, **Habit**: A small or middle-sized glabrous tree, with a crooked trunk, bark grey in colour and smooth in nature. **Leaves**- broadly elliptic, in nature and rounded at the apex, emarginate, with waved margins and very close lateral nerves, **Flowers**- Pure white in colour, fragrant, arranged in flowered racemes, concave, petals 4, oblong, obtuse, and spreading with numerous stamens **Fruit**– globose brown in colour.

# <section-header>

Showing Tree with Flowers of Punnaga







Showing Flowers, Stamens, Flower Buds & Fruits

### *Dillenia Pentagyna* Roxb <sup>[5]</sup> Family –Dilleniaceae

Habitat: Widely distributed in hilly areas of North East states of India and Bangladesh. Habit: Tall deciduous tree grows upto15m. Bark: Greyish white in colour with peeling off in to flakes. Leaves: Simple, alternate, clustered at ends; petiole; apex acute or acuminate, margin denticulate with villous teeth, pubescent on midrib and nerves beneath; Flower: Yellow in colour, fascicled on old leafless branches. Fruit and Seed: Globose with indehiscent carpels, fleshy, covering with thickened fleshy sepals; seeds 1 or 2 per carpel.

### **Aims and Objectives**

- 1) To study the macroscopic and microscopic characteristic features of flower of *Mesua ferrea* Linn. and *Cinnamomum tamala* Nees & Ebern.
- 2) Qualitative analysis of *Mesua ferrea* Linn. and *Cinnamomum tamala* Nees & Ebern.

### **Materials and Methods**

1) *Mesua ferrea* Linn is collected as a fresh form from Mangalore and *Cinnamomum tamala* Nees & Ebern is obtained from the local market.

2) Pharmacognostic evaluation and Morphological identification based on API guidelines.

# Phytochemical Analysis of Different Varieties if Nagakesara

### Materials and Methods

I. Tests for Alkaloids<sup>[6]</sup>

**1. Dragondroff's Test:** To 1 ml of the extract, 1 ml of Dragondroff's reagent was added; formation of orange red precipitate indicated the presence of alkaloids.

**2. Wagner's Test:** To 1 ml of the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicated the presence of alkaloids.

**3. Mayer's Test:** To1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of alkaloids.

**4. Hager's Test:** To1ml of the extract, 3 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

### II. Test for Carbohydrates<sup>[7]</sup>

**1. Molisch Test:** To 2 ml of the extract, 1 ml of  $\alpha$ -naphthol solution and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

**2. Fehling's Test:** To 1ml of the extract, equal quantities of Fehling's solution A and B were added, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.

**3. Benedict's test:** To 5ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

### III. Tests for Proteins and Amino Acids<sup>[8]</sup>

**1. Biuret Test:** To 1 ml of the extract, 1ml of 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins.

**2. Xanthoprotein Test:** To 1 ml of the extract, 1ml of concentrated nitric acid was added. A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsequently added; orange colour indicated the presence of aromatic amino acids.

**3. Lead Acetate Test:** To the extract, 1ml of lead acetate solution is added. Formation of a white precipitate indicated the presence of proteins.

**4. Ninhydrin Test:** Two drops of freshly prepared. 0.2% ninhydrin reagent was added to the extract solution and it was then heated. Development of blue colour revealed the presence of proteins, peptides or amino acids.

### **IV. Tests for Phytosterol**

**1. Libermann Burchard Test:** The extract was dissolved in 2 ml of chloroformin a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution turned red, then blue and finally bluish green, indicated the presence of steroids.

**2. Salkowski Test:** Dissolve the extract in chloroform and equal volume of concentrate sulphuric acid. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer represented the steroid components in the tested extract.

### V. Tests of Glycosides

**1. Legal Test:** The extract was dissolved in pyridine and sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour showed the presence of glycosides.

**2. Baljet Test:** To 1 ml of the test extract 1 ml sodium picrate solution was added and the yellow to orange colour revealed the presence of glycosides.

**3. Borntrager's Test:**<sup>[9]</sup> A few ml of dil. HCl was added to 1 ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1 ml of ammonia. The formation of red colour showed the presence of anthraquinone glycosides.

**4. Keller Killiani Test:** The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue, confirmed the presence of glycosides.

### VI. Test for Saponins

**1.** About 1 ml of methanol extract was diluted separately with distilled water to 20ml, and shaken in a graduated cylinder for 15 minutes. 1cm layer of foam indicated the presence of saponins.

### VII. Test for Flavonoids

**1. Shinoda Test:** To 1 ml of the extract, magnesium turnings were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of red colour showed the presence of flavanoids.

### VIII. Test for Tannins and Phenolic compounds

1. To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.

2. To the extract, potassium dichromate solution was added, formation of a precipitate showed the presence of tannins and phenolic compounds.

### IX. Test for Triterpenoids

1. Two or three granules of tin metal in 2ml thionyl chloride solution were dissolved. 1ml of the extract was then added into the test tube. The formation of a pink colour indicated the presence of triterpenoids.

### X. Test for Fixed Oils

**1. Spot Test:** A small quantity of extract was pressed between two filter papers. Oil stains on paper indicated the presence of fixed oils.

**2. Saponification Test:** To 1 ml of the extract few drops of 0.5N alcoholic potassium hydroxide was added along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. The formation of soap or partial neutralization indicated the presence of fixed oils.

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S. No	Test	M. F curnam	O. L curnam	C. T curnam	C. I curnam				
1)	Alkaloids	-	-	-	+				
2)	Carbohydrates	+	+	-	+				
3)	Tannins	+	+	+	+				
4)	Proteins	-	-	-	-				
5)	Starch	-	-	+	-				
6)	Steroids	+	+	+	-				

### Table 1: Results of phytochemical analysis

### Abbreviations

- 1) MF curnam: Mesua ferrea churnam (Nagakesara)
- 2) OL curnam: Ochrocarpus longifolius churnam (Surapunnaga)
- 3) CI curnam: *Calophyllum inophyllum* churnam (*Punnaga*)
- 4) CT curnam: Cinnamomum tamala (Tamalpatra)

### Table 2: Comparing different morphological characters of Nagakesara

Morphological characters	Mesua ferrea	Ochrocarpus Ionifolius	Calophyllum inophyllum	Cinnamomum tamala	Dillenia pentagyna
Large tree	+	+	-	-	+
Flowers fragrant	+	+	+	-	-
Petals Hooded	+	+	+	-	-
Numerous stamens and golden coloured	+	+	+	-	-
Fruit pitcher shaped	+	+	-	-	-

### Pharmacognostic Study of Nagakesara

Pharmacognostic study was carried out for two species of *Nagakesara* i.e., *Mesua ferrea* Linn. stamens and *Cinnamaomum tamala* Nees and Ebern Buds to find out its source.

### Mesua ferrea



Mesua ferrea flower



Showing stamen under microscope having magnification (10x)



Crude drug of *Cinnamomum tamala* flower bud

### Description

- Organoleptic characters
- 1. Colour : Reddish brown
- 2. Odour : Fragrent
- 3. Taste : Astringent
- 4. Texture: Fine

### a) Macroscopic

Macroscopically we can observe Stamens, Petiole, Filament and Persistent stalk.

Reddish brown in colour with persistent petiole, stamen consists of anther and filament, coppery or golden brown, filament united at base forming a fleshy ring, each stamen containing pollen grains, filament which is slender, filiform, soft in nature, brittle.

### b) Microscopic

### Soaking of Dried Flower:

After soaking the given dried flower in normal water or in boiling water (if the dried flower is hard), then these samples are ready for section cutting and observed under microscope.

### **Transverse and Longitudinal Section through Petals**

- The section consists of epidermis which is two layered i.e., upper epidermis and lower epidermis.
- Numerous trichomes were seen on the upper layer of the epidermis and the epidermis constitutes of a thick cuticle.
- Ground tissue consists of rounded collenchymatous cells which present behind the epidermis. sclerenchymatous celled layers were present and was made up of sclerenchymatous fibres.
- In longitudinal section, numerous oil glands were seen.
- Below the ground tissue, next we observe the lower epidermis which was made up of few layers of collenchymatous cells which are round.



### Longitudinal section of Mesua ferrea crude drug through petal

### **Macroscopical characters**

Crude flower sample - globose or round in shape, dark brown to black in colour with short pedicles and having persistent calyx.

### Microscopical characters (Transverse section of Cinnamomum tamala through petal)

The section shows the presence of both upper and lower epidermis,

Secretory cell layer, Stone cell layer, Epidermis and a layer of anthers cells.

The upper epidermis is uniform, elongated laterally and composed of an outer cuticle and shows the absence of stomata.

Cortical region is made with thin walled parenchymatous cells and embedded with numerous stone cells and secretory glands.

Endodermis is well developed and consists of a layer of anther thus showing the epipetalous nature of a flower.

### Transverse section of Cinnamomum tamala (Meissn.) through petals

**Oil Glands** 



Longitudinal section of *Cinnamomum tamala* crude drug



Transverse section of Cinnamomum tamala crude drug



Transverse section of *Cinnamomum tamala* crude drug through petal



Transverse section of Cinnamomum tamala crude drug through petal

### DISCUSSION

Flower buds of *Nagakesara* plant of different species available in the market are taken up for the study. Due to less availability of *Mesua ferrea* Linn., some other species are used as *Nagakesara*. Nearest point of availability is one of the major reasons for Substitution. *Malabaar Nagakesara*: The unripe fruits of *Cinnamomum tamala* Nees & Ebern were sold in the markets of Chennai, which was considered as a substitute and adulterant for *Nagakesara*, which are black in colour, when dried.

The pharmacognostic screening was an important tool in detecting of adulteration and substitution and to resolve confusion and to find the exact source plant of *Nagakesara*.

The phytochemical screening reveals the presence of carbohydrates, tannins and steroids in *Mesua ferrea* Linn and alkaloids, carbohydrates and tannins in *Cinnamomum tamala* Nees & Ebern. This shows the importance of metabolites and medicinal importance of *Nagakesara* samples.

This work can serve a valuable source of information to establish the plant in future study.

### CONCLUSION

- Though the officinal part of Nagakesara is its flower, there arose a controversy in identity in the medieval period due to many reasons like nonavailability, transportation and increased utilization. Then many locally available species occupied the original drug.
- The study was carried out on main and important market drugs of *Nagakesara* are *Mesua ferrea* Linn. and *Cinnamomum tamala* Nees and Ebern.
- In the present pharmacognostic and phytochemical study a detailed morphological review was done through which it was concluded that *Mesua ferrea* Linn. may be the exact source of *Nagakesara*. This conclusion was made depending upon the survey, opinion of the previous authors like Chunekar, K.C. Sharma P. V and API standards. Its wide availability prominent acceptability and

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also the studies taken up both experimentally and clinically by previous authors also supported this view.

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