

A Pharmacognosy Approach to the Botanical Source of a Cinnamon Fruit Traded as *Nāgakeśara* and *Sirunagappu* in Raw Drug Markets

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ABSTRACT

Background: Adulteration and substitution of *Nagakesara* is a well-established fact in the medicinal plant literature though it is referred by the authors of important Ayurvedic texts to cure piles, dysentery, hiccup etc. The botanical identities of controversial sources of *Nagakesara* are flowers of *Mesua ferrea* L. and *Ochrocarpus longifolius* Benth. and Hook. f., *Calophyllum inophyllum* L., *Dillenia pentagyna* Roxb. and perchance their allied species. Though immature fruits of some *Cinnamomum* species are also reported to be sold as *Nagakesara/Sirunagappu* in the crude drug markets there are no pharmacognostic studies available for the identification and analysis of this crude drug. **Materials and Methods:** In this study, marketed *Nagakesara/Sirunagappu* samples were procured from Chennai raw drug market and analyzed as per the standard methodologies for pharmacognostic studies. **Results:** Macro-microscopical characters and chemical identity of the samples of immature fruits were established to derive set of characters for the identification of this marketed material. The proposed results would help in identification of this crude drug while used as substitute drug for the official source. **Conclusion:** The botanical *Nagakesara* or *Sirunagappu* is neither stamen nor flower, but it is fruit of *Cinnamomum* species. As Western Ghats is habitat for several similar looking species of *Cinnamomum*, there is necessity to study the pharmacognostical characters of all species of *Cinnamomum* before concluding the botanical source of this ambiguous raw drug of Ayurveda and Siddha.

Key words: Adulteration, HPTLC, Lauraceae, Market substitute, Quality standards, Siddha.

INTRODUCTION

Even though various documents prescribe standards for drugs of vegetable origin, problem of identity is a threat to the quality of several medicines of traditional systems of medicine. It is a great challenge for a large scale manufacturer of Ayurveda or Siddha formulations as they wholly depend on drugs from market. Due to the overwhelming demand for some less produced raw drugs, crude drug traders always look for an alternate to keep the supply as per demand. Recently, there is increasing trend in entry of new commodities as substitutes for certain raw drugs, the reason may be attributed to high demand and disturbed frequency of the occurrence of the source plant. Identity of marketed drugs is need of the hour when quality of the herbal medicines is seriously considered. *Nagakeshara* is referred by the authors of important Ayurvedic texts to cure piles, dysentery, hiccup etc. The botanical identities of controversial sources of *Nāgakeśara* are flowers of *Mesua ferrea* L. and *Ochrocarpus longifolius* Benth. and Hook. f., *Calophyllum inophyllum* L. and *Dillenia pentagyna* Roxb.¹

In the past, immature fruits of *Cinnamomum* species were also reported to be traded in place of *Nagakeshara* both as a substitute and as an adulterant. Among fruits of *Cinnamomum* species under trade in India, *C. malabattrum* Reinw. is used in *pan* as a substitute for clove buds.² *C. malabattrum* is a close relative of *C. verum* and is a highly variable species occurring in south India and are found in the Western Ghats and adjoining areas. *Kattu karuva* described by Van Rheedee in *Hortus Malabaricus* (1685)³ was nothing but *C. malabattrum*. Species described under *C. iners* by Hooker (1886)⁴ as well as Gamble (1925)⁵ also refer to *C. malabattrum*. This is a moderately sized tree, sometimes attaining larger proportions. Fruit ellipsoid, up to 8 to 10 mm, cup deep, rather fleshy, the base merging to the pedicel, the rim, with persistent thickened tepals of which the apical part drops off, the basal part rounded and pilose.⁶

Sirunagappu is one of the important drugs used in Siddha system of medicine. There is a lot of confusion existing in the botanical source

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of this drug. *Cinnamomum wightii* Meissn. is endemic to Shola forests of Southern Western Ghats in Tamil Nadu (Annamalai, Palani, Nilgiris, Coimbatore, Dindigul) and Kerala (Idukki, Palakkad, Wayanad, Thiruvanthapuram) is a tree, up to 8 m tall with angular young branchlets, fruit is ellipsoid one seeded berry, up to 1.5 cm long; fruiting perianth cup fleshy, obconical with basal remnants lobes. It is claimed that flower buds of *C. wightii* are used as *Sirunagappu* in preparation of Siddha formulation such as *Amukkura curnam*.⁷ Tender fruits including style and calyx are astringent, aromatic, stimulant and carminative, it is also used to treat wounds, fever, intestinal worms, headache and menstrual problems.⁸

The current investigation is planned to explore pharmacognostical identity of a fruit sample which is found commonly in Chennai raw drug markets in the name of *Nagakesara* and *Sirunagappu*.

MATERIALS AND METHODS

Materials

Dried flower buds sold as *Nagakeshara/Sirunagappu* were procured from Chennai market and the macroscopic characters observed referring to The Flora of Presidency of Madras⁵ and a dried specimen (No: 00643) was deposited in museum of CSMRADDI Chennai. The flower bud was used for the study of macroscopic and microscopic characters. Dried powder was examined for physico-chemical analysis, extraction, phyto-chemical study and HPTLC fingerprinting.

Methods

Macro-microscopical study

The fruits were examined for macroscopy as per standard procedures. For microscopic study specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. 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.⁹

Physico-chemical and preliminary phytochemical analysis

Physico-chemical studies like loss on drying at 105°C, total ash, acid insoluble ash, water-soluble ash, alkalinity of water soluble ash, water soluble extractive and alcohol soluble extractive were carried out as per WHO guidelines.¹⁰ A known quantity of dried powder was extracted in Soxhlet with petroleum ether (60-80°C), chloroform, ethyl acetate, alcohol and finally with water successively and tested for different constituents and preliminary phytochemical tests were done as per the standard methods.^{11,12} The fluorescence behavior of the powdered drug in the day light and ultraviolet light were done by moistening the powder in different solution and viewing under the light of different wavelengths in a UV-chamber.

High performance thin layer chromatography

About 4 g of the coarse plant material were successively extracted in Soxhlet apparatus using *n*-hexane, chloroform ethyl acetate and alcohol. The corresponding extracts were filtered, concentrated and made up to 10 ml in a standard flask. 5, 10 and 15µl of this solution were applied on (E. Merck) aluminum plate percolated with silica gel 60 F₂₅₄ of 0.2 mm thickness, CAMAG Linomat IV applicator was used for applying the spot on plates. The plates were developed in a twin trough chamber, saturated with the established solvent system before 30 min, to a distance of 8 cm. The plate was visualized under in UV 254 and 366 nm. The air dried plate was dipped in vanillin-sulphuric acid reagent and then kept in oven at 105°C till the colour of the spots appeared. The photographs were taken.¹³⁻¹⁵

RESULTS AND DISCUSSION

Macroscopy

The sample consists of mixture of 1 to 2 cm long fragments of slender peduncles, globose to round infected buds forming gall-like structures and 0.3 to 0.5 cm long, oval to elliptical fruits which are often detached from the perianth forming cup-like structure on the thalamus; galls can be easily differentiated by the absence of perianth cups formed from accrescent tepals of the flowers; dark chocolate brown externally and light brown internally, encloses elliptical, off-white fruit in it (Figure 1); the galls are astringent in taste and slight in odour while the fruits are astringent-aromatic in taste and fragrant in odour.

Microscopy

Stalk: TS of stalk is roughly triangular in outline, shows an outer single layer of epidermis having 2 to 4 celled cortex with volatile oil cavities and mucilage cells; discontinuous pericycle formed by group of stone cells; 3 major vascular bundles composed of xylem and phloem; and central pith occupied by stone cells (Figure 2a). When a portion of TS is enlarged, there is an epidermis with outer thick wall, coated with cuticle, bearing unicellular short conical covering trichomes; the cortex is formed by tightly packed parenchyma embedding lot of volatile oil cavities and few mucilage cells; pericycle is formed groups of wide-lumen sclereids; phloem shows cells with yellow contents; xylem shows rows of vessels; the central pith region shows lot of sclereids of varying thickness (Figure 2b).

Perianth cup: TS through perianth cup shows an outer epidermis bearing trichomes similar to that of stalk; beneath outer epidermis there are layers of closely packed parenchyma having a few mucilage cells; the ground tissue is formed by few parenchyma and many small groups of wide-lumen sclereids; the inner epidermis does not clearly show the cells but formed by crushed parenchyma cells having groups of thick-walled sclereids in groups; outer to inner epidermis there are masses of remnant tissue may be part of withered inner perianth (Figure 2c).

Fruit wall: TS through wall of the fruit shows an epicarp with outer thick walled cuticle; below the epicarp there is a hypocarp formed by comparatively large sized parenchyma cells with contents; the outer mesocarp shows groups of thick-walled narrow lumen sclereids while the inner mesocarp has large parenchyma without stone cells; a few idioblasts containing some granular contents are embedded in the inner mesocarp regions; the endocarp is formed by small sized parenchyma cells which are very closely packed; tissue of immature seed such as testa and endosperm are also attached to the endocarp, the centre of the immature seed being hollow (Figure 2d).

Macerate: When portion of stalk with attached perianth cup is macerated it shows thick-walled polygonal cells of epidermis of calyx in surface view having few cicatrix; thick-walled unicellular covering bent trichomes with basal cells; idioblasts containing fully swollen mucilage cells attached to normal parenchyma; spherical to elongated volatile oil containing cells; thick-walled parenchyma with sandy crystals; isolated to groups of broad-lumen sclereids of various sizes and shapes; wide lumen tracheidal fibre with pointed ends; elongated sclereidal fibres; scalariform tracheid with pointed ends; and annular vessels (Figure 3a-o). When fruit wall is macerated it shows polygonal epicarp cells with beaded walls; short reticulate tracheids in groups; large idioblasts containing fully swollen mucilage; isolated to groups of narrow-lumen sclereids of various sizes and shapes; elongated sclereidal fibres; tiny narrow lumen stone cells; spirally thickened tracheids and vessels; and fragment of thick-walled cells from epicarp of basal region of fruit (Figure 4a-o).

Powder: Powder shows a few unicellular conical trichomes, parenchyma with idioblasts containing mucilage and volatile oil cells; lot of stone cells/ sclereids of various sizes and shapes; elongated thick-walled sclereidal

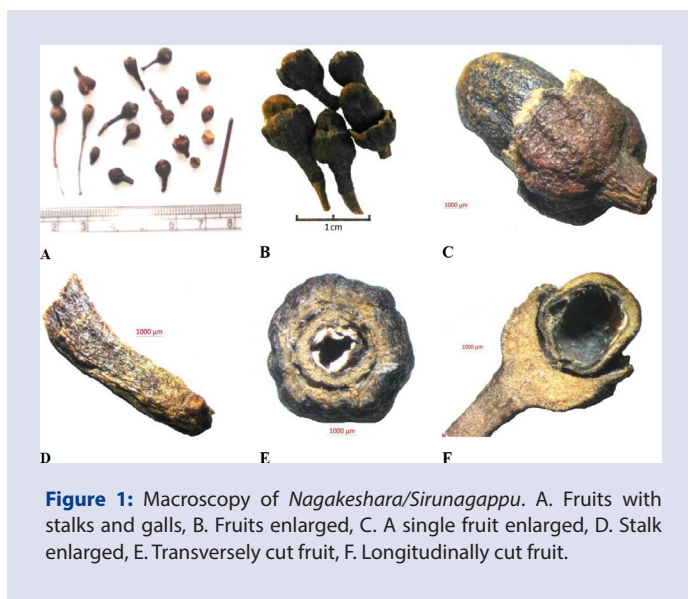


Figure 1: Macroscopy of *Nagakeshara/Sirunagappu*. A. Fruits with stalks and galls, B. Fruits enlarged, C. A single fruit enlarged, D. Stalk enlarged, E. Transversely cut fruit, F. Longitudinally cut fruit.

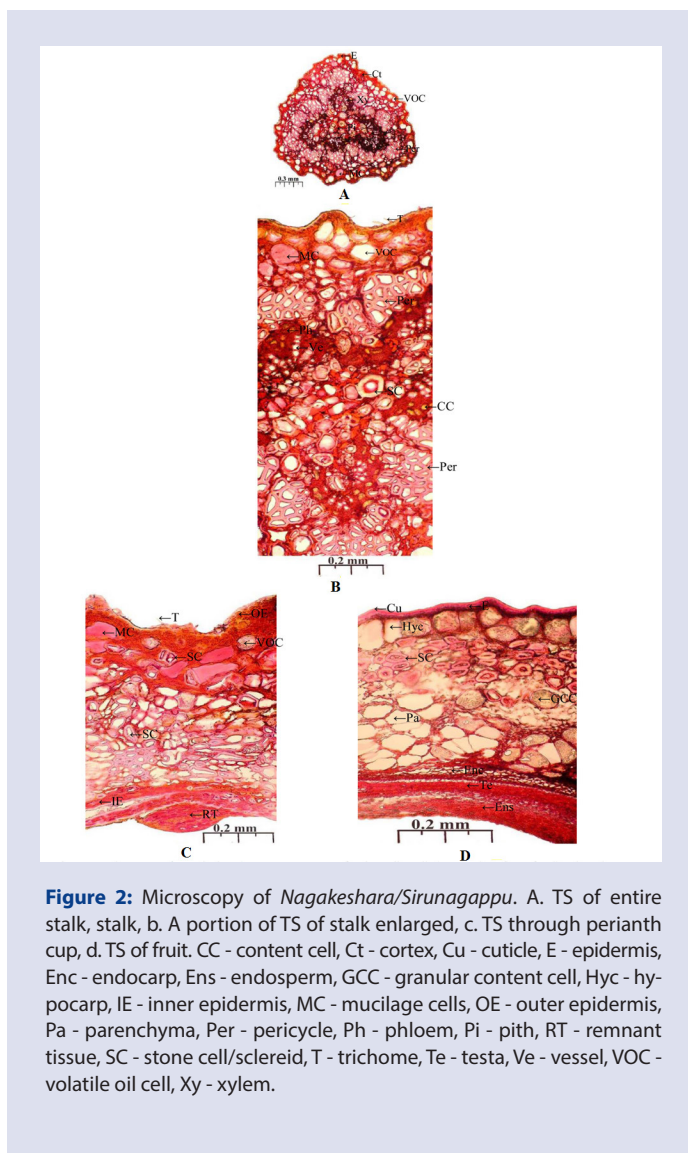


Figure 2: Microscopy of *Nagakeshara/Sirunagappu*. A. TS of entire stalk, stalk, b. A portion of TS of stalk enlarged, c. TS through perianth cup, d. TS of fruit. CC - content cell, Ct - cortex, Cu - cuticle, E - epidermis, Enc - endocarp, Ens - endosperm, GCC - granular content cell, Hyc - hypocarp, Ie - inner epidermis, MC - mucilage cells, OE - outer epidermis, Pa - parenchyma, Per - pericycle, Ph - phloem, Pi - pith, RT - remnant tissue, SC - stone cell/sclereid, T - trichome, Te - testa, Ve - vessel, VOC - volatile oil cell, Xy - xylem.

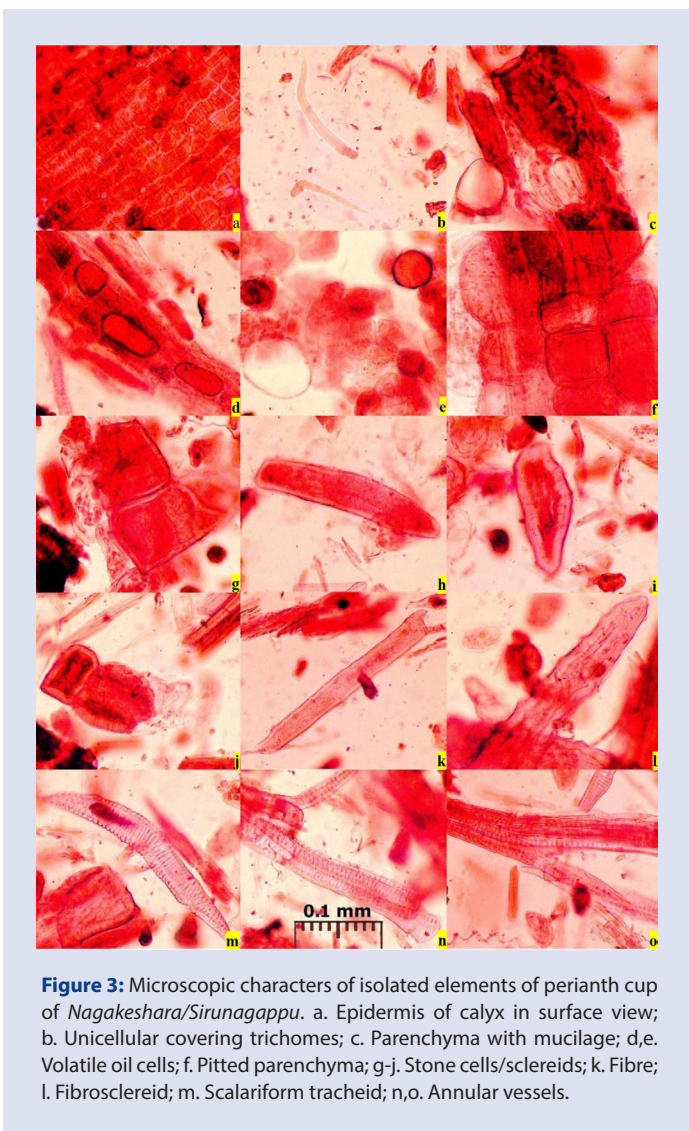


Figure 3: Microscopic characters of isolated elements of perianth cup of *Nagakeshara/Sirunagappu*. a. Epidermis of calyx in surface view; b. Unicellular covering trichomes; c. Parenchyma with mucilage; d,e. Volatile oil cells; f. Pitted parenchyma; g-j. Stone cells/sclereids; k. Fibre; l. Fibrosclereid; m. Scalariform tracheid; n,o. Annular vessels.

fibres; and fragments of vascular bundles with scalariform tracheids (Figure 5); powder is reddish brown in colour with pleasant odour and mucilaginous slightly astringent taste.

Fluorescence behavior

Fluorescence testing is one of the tests used for identification of several herbal drug powders ever since the origin of phytochemistry. When a drug powder is treated with some acidic or alkaline reagent, the phytochemical present in the sample undergo chemical changes which in turn produces some fluorescence behavior when exposed to lights of different wavelength. Examination of the powder of the sample under investigation showed yellow fluorescence with ethanol and methanol, purple fluorescence with acetone and green fluorescence with methanolic/ethanolic NaOH when observed under long UV; other characteristic colour were observed under white light and short UV with different reagents (Table 1). These colour characteristics can be used to detect the raw drug without much expenses in short period of time.

Physico-chemical standards

The physico-chemical parameters such as loss on drying, water soluble extractive, ethanol soluble extractive, total ash, water-soluble ash and

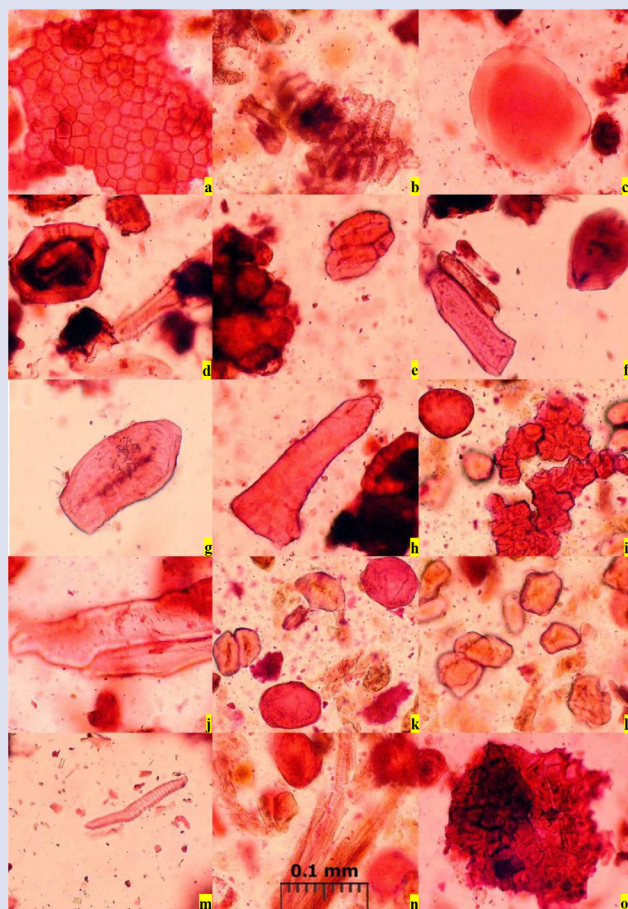


Figure 4: Microscopic characters of isolated elements of fruit wall of *Nagakeshara/Sirunagappu*. a. Epicarp cells in surface view; b. Reticulate parenchyma; c. Mucilage cell; d-h. Sclereids; i. Cells from epicarp of basal region of fruit; j. Sclereidal fibres; k,l. Stone cells; m. Tracheid; n. Spiral vessels; o. Cells from epicarp of basal region of fruit.

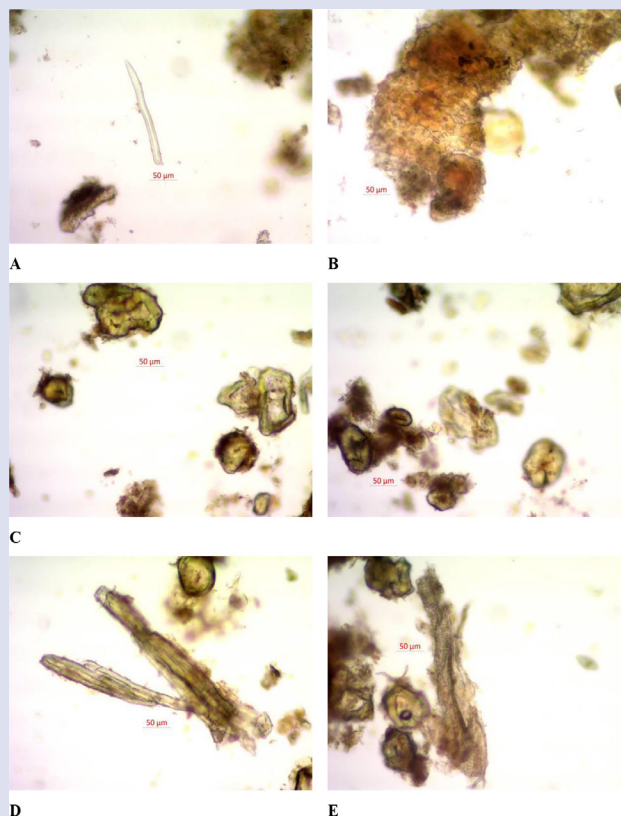


Figure 5: Microscopic characters of powder of fruit with stalk and perianth cup of *Nagakeshara/Sirunagappu*. a. Trichome; b. Parenchyma with mucilage and volatile oil cells; c. Stone cells/sclereids of different sizes and shapes; d. Sclereidal fibres; e. Scleriform tracheids.

acid insoluble ash are important observations towards authenticity and quality affirmation of botanical drugs. Loss on drying the drug was 8.51% revealing the moisture percentage in the drug. Total ash is found to be (3.82%) while acid insoluble and water soluble ash is found to be 1.03 and 0.38% respectively. The water soluble extractive value was 5.76% w/w indicating the presence of readily water soluble polar constituents such as sugars, amino acids and inorganic salts. The ethanol soluble extractive value was 3.41% indicating the percentage of medium polar constituents of the plant material (Table 2).

Preliminary phytochemical tests

The preliminary phytochemical investigation of different extracts of the drug showed the presence of different moieties of phytochemicals. Triterpenoid was present in *n*-hexane, chloroform, ethyl acetate and ethanol extracts; flavanoids were detected in ethyl acetate and ethanol extracts; carbohydrates were present in ethanol extract; quinines, tannin and phenolic compounds were present in ethyl acetate and ethanol extracts; furan was detected from chloroform, ethyl acetate and ethanol extracts (Table 3). The medicinal activity of an herbal drug is due to the presence of these metabolites and these phytochemical moieties are helpful in proposing probable action of the plant drug.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC fingerprinting revealed the presence of various phytoconstituents with their respective R_f values and the photo documentation of the plates showed numerous bands under short UV, long UV and after derivatisation. Vanillin-sulphuric acid reagent was used as visualizing agent to effect visualization of the resolved spots. *n*-Hexane, chloroform, ethyl acetate and ethanol extracts were fingerprinted using solvent system toluene and ethyl as mobile phase. Extracts showed different banding pattern useful for identification of different extracts under different visualization techniques. *n*-Hexane extract showed 8, 6 and 8 spots under short UV, long UV and after derivatization respectively (Figure 6a); chloroform extract showed 6, 11 and 10 spots under short UV, long UV and after derivatization respectively (Figure 6b); ethyl acetate extract showed 9, 7 and 6 spots under short UV, long UV and after derivatization respectively (Figure 6c); and ethanol extract showed 3, 7 and 4 spots under short UV, long UV and after derivatization respectively (Figure 6d). As ethyl acetate extract showed good fingerprint pattern on photo-documentation it was chosen for HPTLC studies. Densitometric scan at 620 nm (post derivatization) showed 18 peaks with R_f 0.05 (8.44%), 0.08 (0.93%), 0.16 (3.05%), 0.20 (0.74%), 0.25 (7.17%), 0.27 (3.63%), 0.31 (4.56%), 0.39 (0.72%), 0.44 (1.18%), 0.46 (0.67%), 0.50 (1.01%), 0.57 (1.02%), 0.62 (3.36%), 0.66 (0.85%), 0.73 (4.25%), 0.77 (6.78%), 0.81 (2.85%) and 0.86 (8.79%) (Table 4). HPTLC is an identity test proposed in most of standardization documents for herbal drugs. As using HPTLC is simple, cost effective and rapid detection of herbal drugs

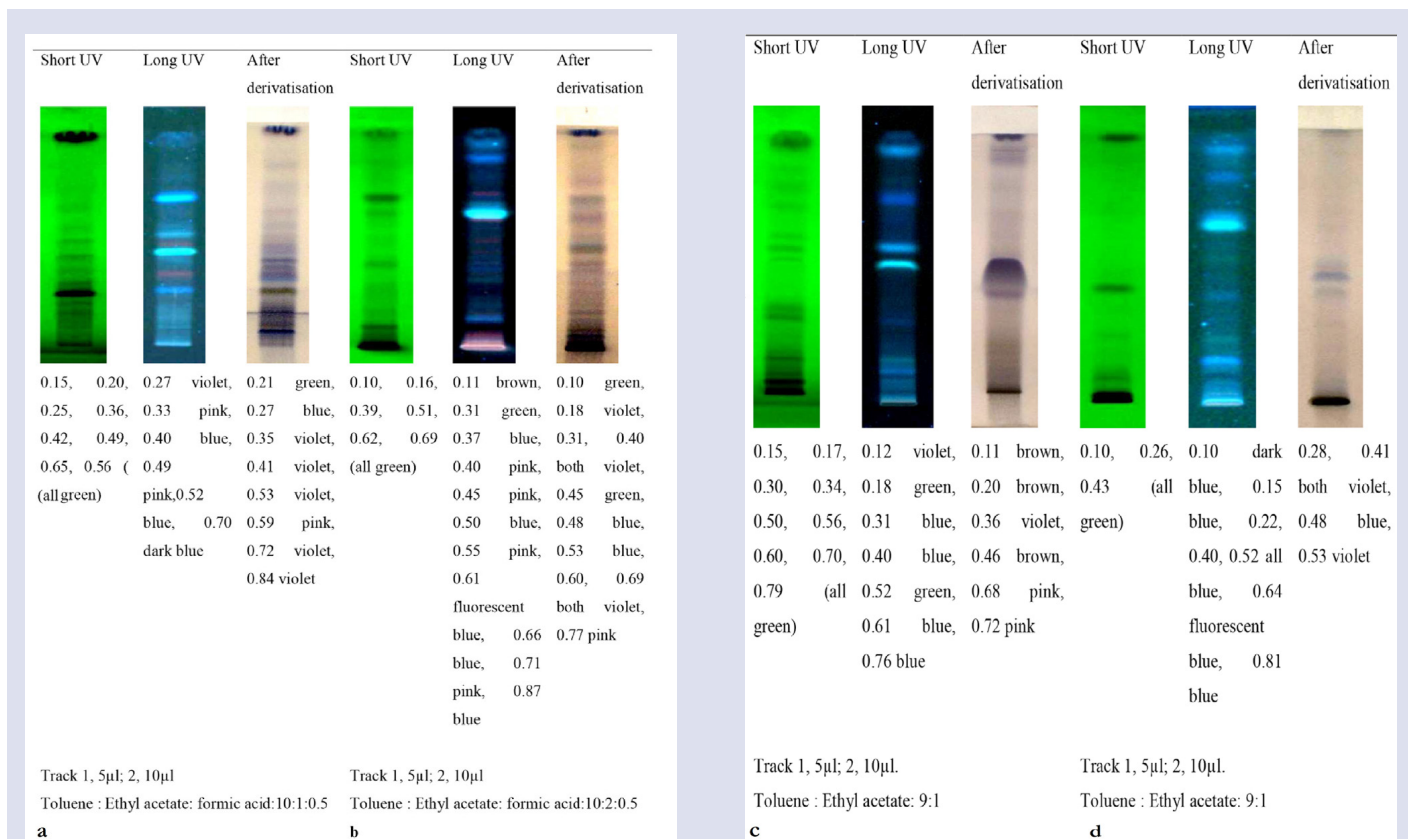


Figure 6: TLC photo-documentation of different extracts of *Nagakesara/Sirunagappu*. a. n-hexane extract; b. Chloroform extract; c. Ethyl acetate extract; d. Ethanol extract.

Table 1: Fluorescence behavior of *Nāgakesāra*.

Powder	Visible light	254 nm	366 nm
As such	Brown	Black	No fluorescence
With methanol	Brownish yellow	Dark brown	Yellow fluorescence
With acetone	Golden brown	Dark brown	Purple fluorescence
With ethanol	Brown	Dark brown	Yellow fluorescence
With dil. Ammonia	Reddish brown	Brown	No fluorescence
With 1N methanolic NaOH	Yellow	Brown	Green fluorescence
With 1N ethanolic NaOH	Pale yellow	Brown	Green fluorescence
With 1N HCl	Brown	Black	No fluorescence
With 50% H ₂ SO ₄	Brown	Black	No fluorescence
With 50% HNO ₃	Brown	Black	No fluorescence
With 50% KOH	Brown	Reddish-brown	No fluorescence

Table 2: Physico-chemical parameters of *Nāgakesāra*.

Parameter	% w/w (Mean ± SEM)
Loss on drying	8.505 ± 0.16
Water soluble extractive	5.755 ± 0.49
Alcohol soluble extractive	3.415 ± 0.47
Total ash	3.82 ± 0.10
Water soluble ash	0.38 ± 0.24
Acid insoluble ash	1.03 ± 0.20

this chromatographic technique is more in use as identity test unlike other chromatography. Standardization of fingerprint for the drug has been performed in this study for quick identity and quality control.

An earlier report on similar source of crude reported recently claimed the botanical source the drug to be *Cinnamomum wightii* Meissn. flowers.¹⁶ However current study clearly shows that the sample are fruits of some *Cinnamomum* species. In south India there are no scrupulous collections from one particular species of *Cinnamomum* for this purpose. Many similar looking species of *Cinnamomum* such as *C. zeylanicum*, *C. malabattrum*, *C. sulphuratum*, *C. wightii* are collected from wild and traded as

Table 3: Preliminary phytochemical test for different solvent extracts of *Nāgakesāra*.

Constituents	<i>n</i> -Hexane	Chloroform	Ethyl acetate	Ethanol
Steroid	-	-	-	-
Triterpenoid	+	+	+	+
Flavonoid	-	-	+	+
Furan	-	+	+	+
Sugar	-	-	-	+
Coumarin	-	-	-	-
Quinone	-	-	+	+
Alkaloid	-	-	-	-
Tannin	-	-	+	+
Phenol	-	-	+	+
Acid	-	-	-	-
Saponin	-	-	-	-

Present (+), Absent (-)

Table 4: HPTLC of ethyl acetate extracts of *Nāgakesāra*.

Peak	R _f Value	Area %
1	0.05	8.44
2	0.08	0.93
3	0.16	3.05
4	0.20	0.74
5	0.25	7.17
6	0.27	3.63
7	0.31	4.56
8	0.39	0.72
9	0.44	1.18
10	0.46	0.67
11	0.50	1.01
12	0.57	1.02
13	0.62	3.36
14	0.66	0.85
15	0.73	4.25
16	0.77	6.78
17	0.81	2.85
18	0.86	8.79

Sirunagappu or *Nagakesara*. Owing to similarity in organoleptic features, same type of confusion already exists about botanical species of Cinnamon bark drugs and leaf drugs too. The pharmacognostical studies are the first and foremost study required on every herbal drug before any further studies are taken. This primary analysis, though are not so sophisticated, are important in herbal drug research while considering adulteration and substitution.

The term *Nagakesara* in Sanskrit is connected to stamen; hence the source of it must be stamen of flowers. From this fact it can be considered that stamens of *Mesua ferrea* or some other flower similar to *M. ferrea* must be the drug. The term *Sirunagappu* in Tamil connects with a flower, hence a flower must be the source of the drug. One of the flower sources

of *Sirunagappu/Nagakesara* is *Ochrocarpus longifolius*, hence the same may be considered as *Sirunagappu*. The drug studied in the current study belongs genus *Cinnamomum*, but the drug is sold in market as immature fruits not flowers. If *Cinnamomum* species is to be used as *Sirunagappu* then the drug must originate from a flower not fruit. Or the drug name could be a misnomer like fruit of *Cuminum cyminum* is called as cumin seed.

CONCLUSION

Macro-microscopical and physico-chemical fingerprint standards for the fruit of *Cinnamomum* species traded in the name of *Nagakesara* or *Sirunagappu* is a mixture of immature fruits of many commonly occurring *Cinnamomum* species of Western Ghats. There are several species of *Cinnamomum* yielding similar aromatic fruits hence there is need to study flowers and fruits of all the *Cinnamomum* species occurring in Western Ghats before concluding the botanical source of *Sirunagappu* to be *C. wightii*. The study also concludes that the botanical *Nagakesara* or *Sirunagappu* is neither stamen (as suffixed by *Kesara* meaning stamen nor flower (as suffixed by *Poov* meaning flower), but it is immature fruits of *Cinnamomum* species.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CC: content cell, **Ct:** cortex, **Cu:** cuticle, **E:** epidermis, **Enc:** endocarp, **Ens:** endosperm, **GCC:** Granular Content Cell, **Hyc:** hypocarp, **IE:** inner epidermis, **MC:** mucilage cells, **OE:** Outer Epidermis, **Pa:** parenchyma, **Per:** pericycle, **Ph:** phloem, **Pi:** pith, **RT:** Remnant Tissue, **SC:** Stone Cell/sclereid, **T:** trichome, **Te:** testa, **Ve:** vessel, **VOC:** Volatile Oil Cell, **Xy:** xylem.

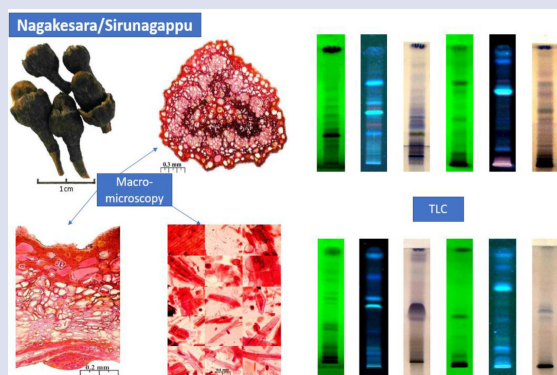
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GRAPHICAL ABSTRACT



SUMMARY

- Macro-microscopical and physico-chemical fingerprint standards for the fruit of *Cinnamomum* species traded in the name of Nagakesara or Sirunagappu is a mixture of immature fruits of many commonly occurring *Cinnamomum* species of Western Ghats. There are several species of *Cinnamomum* yielding similar aromatic fruits hence there is need to study flowers and fruits of all the *Cinnamomum* species occurring in Western Ghats before concluding the botanical source of *Sirunagappu* to be *C. wightii*. The botanical *Nagakesara* or *Sirunagappu* is neither stamen (as suffixed by Kesara meaning stamen nor flower (as suffixed by *Pooov* meaning flower), but it is immature fruits of *Cinnamomum* species.

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