ORIGINAL ARTICLE

Identification of phytoconstituents of Cirunakappu by gas chromatography–mass spectrometry

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Abstract

Introduction: Cirunakappu is the regional name of *Cinnamomum wightii* (flower bud) which is also known as Nagakesaram in Tamil. The flower bud of *C. wightii* is used in many Siddha formulations. The aim of this communication is to identify the phytoconstituents of few column chromatographic fractions of the ethanolic extract of Cirunakappu through gas chromatography–mass spectrometry (GC–MS). **Materials and Methods:** Cirunakappu flower bud was coarsely powdered, extracted with ethanol at room temperature (yield: 184 g). This extract was subjected to column chromatography over silica gel and eluted with hexane and mixture of hexane and chloroform in increasing polarities. Fraction I eluted with hexane-chloroform (98:2), fraction II eluted with hexane-chloroform (90:10), fraction III eluted with hexane-chloroform (80:20), and fraction IV eluted with hexane-chloroform (50:50) were subjected to GC–MS using the HP 5 MS column of 30 m × 0.25 mm ID and 0.25 μm film thickness analysis. **Results and Discussion:** Fraction I yielded six peaks in which linalool, γ-muurolene, α-cadinol, γ-sitosterol, and n-hexadecanoic acid were identified. The fraction II separated 15 peaks, among which eugenol, δ-cadinene, epiglobulol, cadina-1,4-diene, triacontyl acetate, and 3,5-bis-tert-butylphenol were identified. Fraction III showed 15 peaks: α-copaene, cetene, (+)-epibicyclo-sesquiphellandrene, tetradecene, 1-octadecene, 1,2-dimethylcyclo hexadecane, 1-docosene, cyclotetracosane, and 1-nanodecene were identified. From fraction IV, γ-sitosterol, cetene, 1,2-diethyl-cyclohexadecane, 1-tetradecene, 1-eicosene, cyclotetracosane, 1-nanodecene, 1-octadecene, 2,4-bis-(1,1-dimethylethyl) phenol, and 1-methyl-2-pyrrolidinone were identified. **Conclusion:** These chemicals are 1st time explored from this plant.

Key words: *Cinnamomum wightii,* Cirunakappu, gas chromatography–mass spectrometry, Nagakesaram, Nagakesara

INTRODUCTION

Cirunakappu is a medicinal flower bud used in many Siddha formulations. It is carminative, astringent, acrid, purgative, diaphoretic, and aromatic. Cirunakappu finds use in many Siddha formulation, namely, Amukkarac curanam, Civataic curanam, Elatic curanam, Ilaku Cantanatit Tailam, Incic curanam, Kantaka Racayanam, Karicalai Ilakam, Narattai Ilakam, Parankippattai Iracayanam, Talicatic curanam, Talicativatakam, Vilvati Ilakam,[1] Ilankathi curanam, Kadukkai Ilakam, Chandrakanthi Curanam, Naavalpattai Nei, Pooranathi Ilakam, Mahavilvathi Ilakam, MilaguthTailam, Megathennai,[2] etc. It is botanically equated to *Mesua nagassarium* (Burm.f.) Kosterm. in classical Siddha literature. It is known as Ceylon Iron wood in English, Nagakesara in Sanskrit, Telugu, and Kannada, Nagkesar in Hindi, and Nakappuvu in Malayalam. The flower is included in curanam, legiyam, and tailam for flavoring since is aromatic. The dried flower is ground to a paste after frying with cow's ghee and applied on the legs for relief from burning sensation. The flower is fried in ghee and powdered to curanam consistency. A 1–2 g of this curanam with ghee as vehicle cures bleeding, dryness of mouth, nagging pain in stomach, indigestion, excessive sweating.[3] Although Cirunakappu is botanically

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called as *M. ferrea*, in the crude drug market of Tamil Nadu, in the name of Cirunakappu/Nagakesara, *M. ferrea* is not sold, *Cinnamomum wightii* is sold.[4] *Cinnamomum ovalifolium* Gardner ex Meisn. is synonym to *C. wightii.*[5] The genus Cinnamomum belongs to the family, Lauraceae comprising evergreen aromatic trees and shrubs. The leaves and bark are a rich source of aromatic oils. Over 250 species of the genus are distributed in South Asia, China, and Australia. Twenty-six species of Cinnamomum are found in India, of which 12 are from Northeast and South India. Trees of Cirunakappu are found mainly in the Shola forest of South India and also in the Western Ghats.[6] Previously, in the review on the aroma profile of *Cinnamomum* species, namely, *C. tamala, C. verum, C. pauciflorum, C. champokianum. C. glaucescens, C. impressinervium, C. camphora, C. bejolghota, C. sulphuratum,* and *C. glanduliferum,* many compounds are reported which were identified by gas chromatography–mass spectrometry (GC–MS).[7] From *C. wightii* flower bud, no chemical constituent is reported, and hence, authors aimed collect Cirunakappu from market and subjected to phytochemical study. In this communication, the chemicals have been identified from few earlier fractions of ethanol extract through GC–MS.

MATERIALS AND METHODS

Plant Procurement

Dried flower buds of Cirunakappu (5 kg) were procured from Chennai market.

Extraction

Cirunakappu flower buds (5 kg) were coarsely powdered, were extracted with ethanol at room temperature, which yielded 184 g of crude extract.

Column Chromatographic Fractionation

This extract (50 g) was subjected to column chromatography over silica gel (100–200 mesh) and eluted with hexane and with Chloroform in increasing polarities. Fraction I (78–149) eluted with hexane:chloroform (98:2), fraction II (150–261) eluted with hexane-chloroform (90:10), fraction III (334–361) eluted with hexane-chloroform (80:20), and fraction IV (407–428) eluted with hexane-chloroform (50:50) were combined after the TLC verification then the combined fractions subjected to GC–MS.

GC–MS Analysis

GC–MS analysis of the combined fractions of Cirunakappu was performed using a Agilent (Instrument Model: 7890 A, MS 5975) and a gas chromatograph interfaced to a mass spectrometer (GC–MS) equipped with a capillary column (HP 5 MS: 30 m × 0.25 mm ID × 0.25 µm film thickness). For GC–MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed with an average velocity of 35 cm/sec. The injector temperature was maintained at 250°C. The starting temperature of the oven was kept at 50°C and programmed to 100°C with the increment of 25°C/min and to 300°C with the increment of 5°C/min with 1 min hold time. Mass spectra were taken at an electron energy of 70 eV. The total GC/MS running time was 42 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Moreover, the software adopted to handle mass spectra and chromatograms was a MSD Chemstation.

Identification of Phytoconstituents

Interpretation on mass spectrum GC–MS was carried out using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Natural essential oils are usually mixtures of terpenoids (mainly monoterpenoids and sesquiterpenoids), aromatic compounds, and aliphatic compounds. As mass spectra of these compounds are usually very similar, peak identification often becomes very difficult and sometimes impossible. To address the qualitative determination of composition of complex samples by GC–MS and to increase the reliability of the analytical results, it is necessary to utilize retention indices identities.[8] Flower bud of *C. wightii* Meisn. is considered as Nagakesara/Cirunakappu and is sold in the name of Black Nagakesara/Karu/Sirunagappu. This is widely used by the Siddha practitioners in the state. Earlier researchers reported that the Nagakesara which is sold in Chennai crude drug market is *C. wightii* and it is also called as Sirunagappu in Tamil.[9] The GC–MS spectra of fraction 1–4 are shown in Figures 1-4 and identified compounds are listed in Tables 1-4.

The flower bud of *C. wightii* is the medicinally useful part. However, in the market, immature fruits are sold.[10] Suppliers are uneducated and not aware of their spurious supply. Major reasons are confusion in name, non-availability, and lack of data about authentic plant. Even scientific community and traditional physicians are unaware of it. Dried floral buds of *Mesua ferrea* Linn., dried fruits of *Dillenia pentagyna* Roxb.

and dried fruiting inflorescence of *C. wightii* Meisn., are used as Nagakesara in several regions of India.[11] For present in investigation, Nagakesara were purchased from Chennai market. However, on the microscopic examination, it was

Table 1: List of phytoconstituents from fraction 1 identified by GC–MS

RT	Name of the compound	CAS No.	Molecular formula	MW (g/mol)	Retention index	Peak area (%)
4.295	Linalool	78-70-6	$C_{10}H_{18}O$	154.25	1104	2.81
8.279	γ-Muurolene	30021-74-0	$C_{15}H_{24}$	204.35	1477	56.90
8.442	α–Cadinol	481-34-5	$C_{15}H_{26}O$	222.37	1673	26.48
12.414	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	256.40	1971	4.29
31.919	γ-Sitosterol	83-47-6	$C_{29}H_{50}O$	414.70	3351.3	5.72

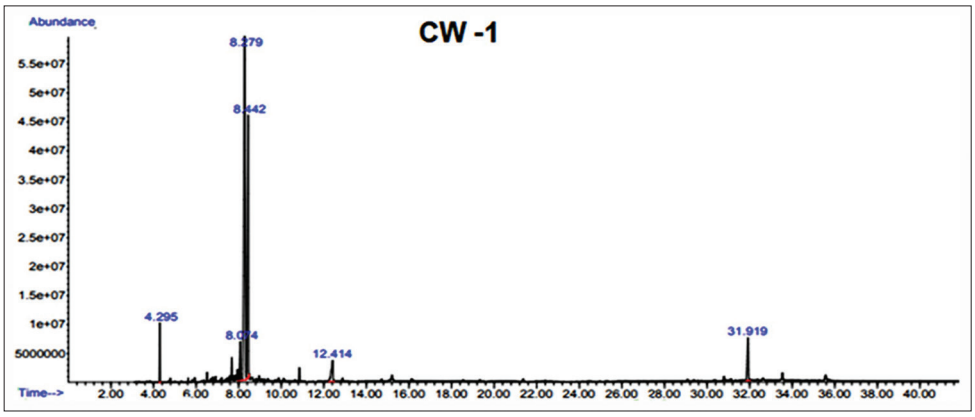

Figure 1: GC-MS spectra of fraction 1

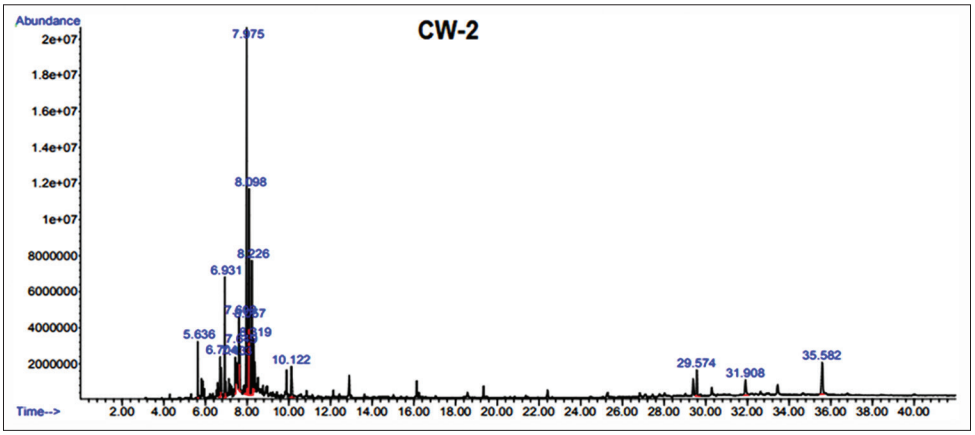

Figure 2: GC-MS spectra of fraction 2

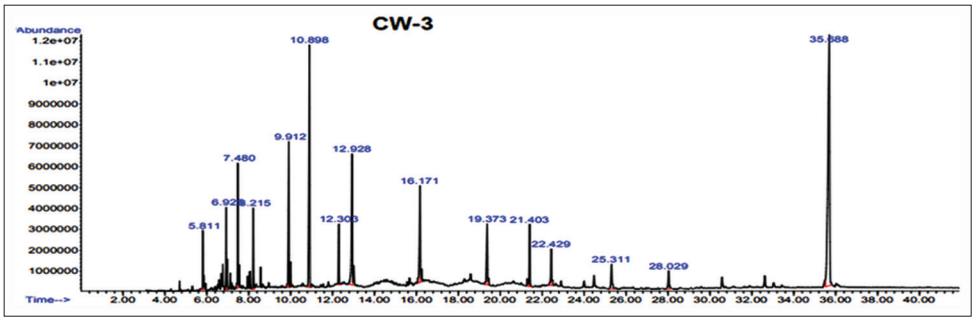

Figure 3: GC-MS spectra of fraction 3

Table 2: List of phytoconstituents identified from fraction 2 by GC–MS

RT	Name of the compound	CAS No	Molecular formula	MW	Retention index	Peak area (%)
5.636	Eugenol	97-53-0	$C_{10}H_{12}O_2$	164.2	1373	2.78
6.704	γ-Cadinene	483-76-1	$C_{15}H_{24}$	204.35	1519	7.27
6.931	Cadine1,4-diene	16728-99-7	$C_{15}H_{24}$	204.35	1539	13.71
7.433	Epiglobulol	88728-58-9	$C_{15}H_{26}O$	222.36	1608	2.31
8.098	3,5-bis-[tert-butylphenol]	1138-52-9	$C_{14}H_{22}O$	206.32	2328	2.68
31.968	Triacontyl acetate	41755-58-2	$C_{32}H_{64}$	480.84	3398.06	2.27

Table 3: List of phytoconstituents from fraction 3 identified by GC–MS

RT	Name of the compound	CAS-No	Molecular formula	MW	Retention time	Peak area %
5.811	α-Copaene	360-33-0	$C_{15}H_{24}$	204.36	1376	2.30
6.925	1-Tetradecene	1120-36-1	$C_{14}H_{28}$	196.37	1389	1.92
7.480	(+)Epibicyclo-sesquiphellandrene	54324-03-7	$C_{15}H_{24}$	204.35	1521	3.80
8.215	Cetene	629-73-2	$C_{16}H_{24}$	224.43	1587	5.11
9.912	1-Octadecene	112-88-9	$C_{18}H_{36}$	252.47	1795	7.55
10.898	1-Nonadecene	18435-45-5	$C_{19}H_{38}$	266.55	1894	1.47
12.928	Cyclohexadecane 1,2-diethyl	155-85-3	$C_{20}H_{40}$	280.54	2071	8.90
16.171	1-Docosene	1599-67-3	$C_{22}H_{44}$	308.56	2194	6.70
28.029	Cyclotetracosene	297-03-0	$C_{24}H_{48}$	336.68	2589	4.12

Table 4: List of phytoconstituents from fraction 4 identified by GC–MS

RT	Name of the compound	CAS No	Molecular formula	MW	Retention index	Peak area%
4.021	1-Methyl-2-pyrrolidinone	872-50-4	C_5H_9NO	99.13	1045	5.39
5.811	1-Tetradecene	1120-36-1	$C_{14}H_{28}$	196.37	1389	3.16
6.692	2,4-bis (1,1-dimethyl ethyl)Phenol	96-76-4	$C_{14}H_{22}O$	206.32	1513	4.09
7.464	Cetene	629-73-2	$C_{16}H_{24}$	224.43	1587	5.23
8.366	Cyclotetracosene	295-17-0	$C_{14}H_{28}$	196.37	1663	3.72
9.889	1-Octadecene	112-88-9	$C_{18}H_{36}$	252.47	1795	6.33
11.796	1-Nonadecene	18435-45-5	$C_{19}H_{38}$	266.55	1894	2.89
12.898	1-Eicosene	3452-07-1	$C_{20}H_{40}$	280.53	1996	5.49
16.136	1,2-diethyl cyclohexadecane	155-85-3	$C_{20}H_{40}$	280.54	2071	6.80
31.884	γ-Sitosterol	83-47-6	$C_{29}H_{50}O$	414.70	3351.3	4.85

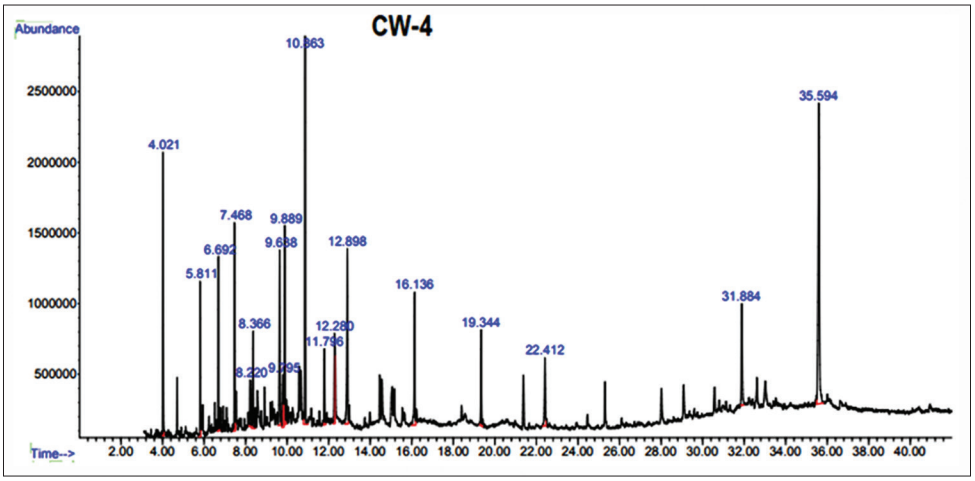

Figure 4: GC-MS spectra of fraction 4

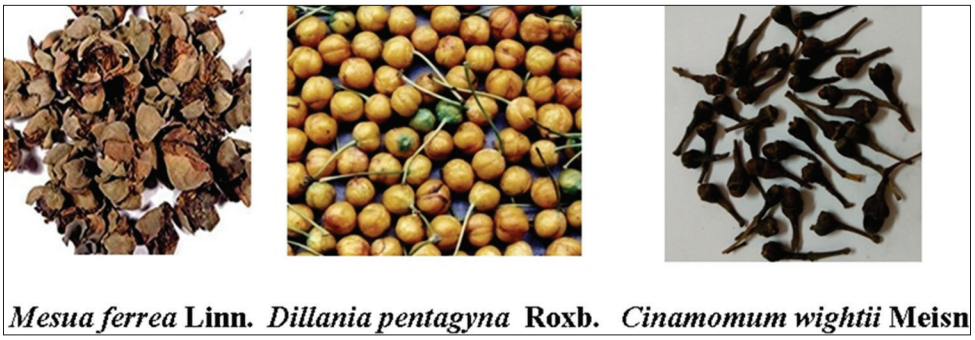

Figure 5: Picture of Nagakesara (*M. ferrea*), Chennai Nagakesara (*C. wightii*), and Malabar Nagakesara (*Dillenia pentagyna*)

found to be *C. wightii*. The picture of Nagakesara (*M. ferrea*), Chennai Nagakesara (*C. wightii*), and Malabar Nagakesara (*Dillenia pentagyna*) is shown in Figure 5.

The GC–MS results showed that the presence of linalool, γ-cadinene, cadine-1, 4-diene, α-copaene, cetene, α-cadinol, 1-tetradecene, and eugenol. This is 1st time report in *C. wightii*.

These phytoconstituents were already reported in Cinnamomum genus: Linalool from *C. glanduliferum,*[12] *C. sulphuratum,*[13] and *C. zeylanicum*;[14] γ-cadinene from *C. zeylanicum*;[15,16] cadine-1,4-diene, α-copaene, cetene, α-cadinol, and 1-tetradecene from *C. zeylanicum*;[17] and eugenol from *C. glanduliferum*,[12] *C. zeylanicum,*[18-21] and *C. sulphuratum.*[22]

As per the earlier authors, the market sample of Chennai Nagakesara is reported to be a mixture of Cinnamomum species.[10] The result of the present study reveals that the compounds were already reported from *C. glanduliferum, C. zeylanicum,* and *C. sulphuratum.* Hence, the investigated plant may be a mixture of *C. glanduliferum, C. zeylanicum, C. sulphuratum,* and *C. wightii.* Otherwise, all the reported compounds may be from *C. wightii* alone. Because γ-muurolene, γ-cadinene, epiglobulol, (+)-epibicyclo-sesquiphellandrene, γ-sitosterol 3,5-bis-[tert-butylphenol], cyclotetracosene, 1-methyl-2-pyrrolidinone, 2,4-bis(1,1-dimethyl ethyl) phenol, triacontyl acetate, 1-docosene, cyclotetracosene, n-hexadecanoic acid, 1-octadecene, 1-nonadecene, 1-eicosene, and 1,2-diethyl cyclohexadecane compounds were not reported from any of the species of Cinnamomum.

CONCLUSION

Wild samples of *C. wightii* flower bud as well as immature fruit have to be collected as per good collection practices and be reinvestigated for the confirmation of the species which is sold in the name of Cirunakappu in the market.

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