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Chemical Fingerprint of Leaves of *Cinnamomum sulphuratum* Nees Growing in Kodagu, Karnataka

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ABSTRACT

Tamalapatra (leaves of *C. tamala*) is one among such important commodity used in the preparation of many Ayurvedic medicines. Leaves of *C. sulphuratum* are sometimes used as substitute to tamalapatra as a flavoring agent. Comprehensive chemical fingerprint profile is useful in the identification of any plant material. This investigation is an attempt to fingerprint the chemical characteristics of leaf of *C. sulphuratum*. Leaves from plants of *C. sulphuratum* growing wildly in Kodagu district were collected and subjected to physico-chemical, HPTLC and GC-MS analysis as per standard procedures. Physico-chemical constants, HPTLC fingerprint and essential oil composition were documented. The chemical fingerprint established in the present study will serve the purpose of standardization of this leaf drug, volatile oil composition by GCMS will be a diagnostic test for differentiation of leaves of *C. sulphuratum* from other *Cinnamomum* Sp.

Keywords: Kodagu, GC-MS, HPTLC, *tamalapatra*.

1. Introduction

Cinnamomum is a genus of the family Lauraceae covering many spices. Leaves of different species of *Cinnamomum* are used as substitute to *tamalapatra* [1-4]. On account of easy availability and similarity in flavor, different parts of *Cinnamomum sulphuratum* are in use as substitutes for commercial Cinnamon derived spices. It is an evergreen tree up to 8 m tall, found in the southern Western Ghats of India. It is one of the 12 endemic south Indian species of *Cinnamomum* [5] but it is also reported from North Cachar Hills of Assam, Northeast India [6]. Existence of four chemotypes of *C. sulphuratum* such as linalool-type [7], citral and cinnamaldehyde-type [8], methyl cinnamate-type [9] and cinnamaldehyde-type [10] have been reported from Northeast India. A benzyl benzoate rich natural chemotype has been reported from the southern Western Ghats [11]. In this communication we attempt to report the chemical composition of *C. sulphuratum* from Kodagu district of Karnataka. Kodagu, also known by its anglicized former name of Coorg, occupies an area of 4,102 square kilometers in the Western Ghats of south western Karnataka. It is a hilly district located on the eastern slopes of the Western Ghats, the lowest elevation of which is 900 meters above sea-level. Kodagu has an average temperature of 15 °C, ranging from 11 to 28 °C, with the highest temperatures occurring in April and May [12].

2. Materials and Methods

2.1 Plant Materials

Fresh flowering twigs of *C. sulphuratum* were collected from plants growing wild in Talakaveri of Kodagu district and identified using description available in different floras [13, 14]. Leaves from botanically identified twigs were harvested, and few were shade dried. Fresh leaves were used for extraction of volatile oil. Air dried leaves were powdered and stored in air tight containers for further chemical examination.

4.2 Instrumentation and Techniques

Physico-chemical characters were carried as per the WHO guidelines. Volatile oil of the sample of *C. sulphuratum* were distilled using Clevenger's apparatus [15]. TLC characterization of the *n*-hexane extract was done as per standard procedure [16]. six, twelve and eighteen µl of the extracts were applied on a precoated silica gel F_{254} aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in benzene : ethyl acetate (9.9:0.2) and the developed plates were visualized and scanned under UV 254, 366, under white light and after derivatisation in anisaldehyde-sulphuric acid spray reagent at 620 nm. R_f, colour of the spots, densitometric scan and superimposability of densitogram were recorded [17]. GC-MS of essential oil of *C. sulphuratum* was carried out using Shimadzu gas chromatograph with a SE-30 10 % Chromosorb-W packed stainless steel column (2 m x 2 mm). Oven programme: 60 °C (5 min), 60°-260 °C (5 °C/min), 260 °C (10 min); carrier gas – nitrogen, flow rate 40 ml/min; injector temperature 240 °C; detector temperature 240 °C. Individual components were identified by database of mass spectra matching with literature available in the libraries like NIST and WILEY by comparison of their RT values.

5. Results

Results obtained for the physico-chemical tests are tabulated in Table 1.

Table 1: Physico-chemical parameters *Cinnamomum sulphuratum*

Parameters	Value
Loss on drying at 105 °C % w/w	12.10
pH of water soluble extractive	5.59
Total ash % w/w	4.6
Acid insoluble ash % w/w	0.40
n- Hexane soluble extractive % w/w	2.29
Alcohol soluble extractive % w/w	6.37
Water soluble extractive % w/w	16.32
Swelling factor ml	15

R_f values of the spots and their colour on TLC of *n*-hexane extract are tabulated in Table 2.

Table 2: R_f value of *n*- hexane extract of *Cinnamomum sulphuratum* 18 µl

At 254 nm	At 366 nm	Under white light	Post derivatisation
-	-	0.03 L yellow	-
-	0.11 F purple	-	0.11 Green
0.23 L green	-	-	-
0.29 Green	0.29 F purple	0.29 Green	0.29 Green
-	-	-	0.40 Purple
0.44 l green	0.44 F purple	0.44 L green	0.44L green
-	-	-	0.61 Purple
0.91 Green	-	-	0.91 L green
-	0.94 F violet	-	0.94 L green
0.97 Green	-	-	-

D - Dark; F - fluorescent; L – light

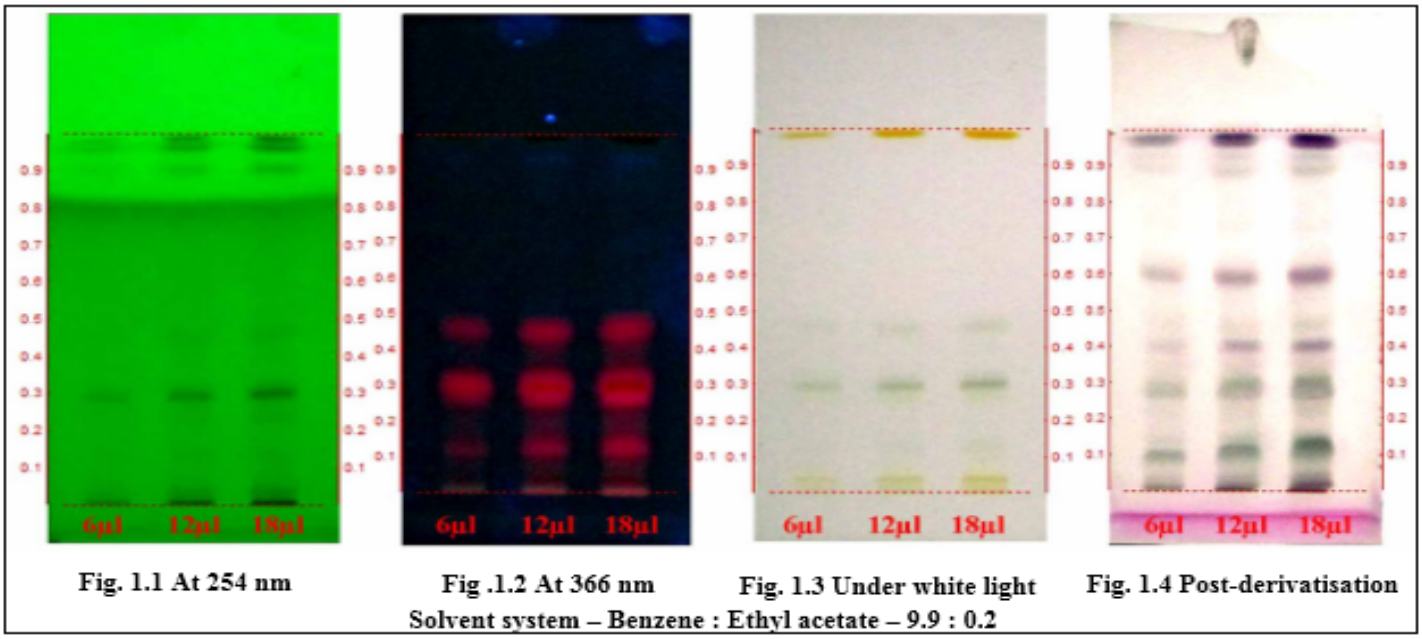

Fig 1: TLC photodocumentation of n-hexane extract of Cinnamomum sulphuratum

HPTLC densitometric scan of *n*- hexane extract of *C. sulphuratum* at UV 254, 366 nm, under white light and after derivatisation with anisaldehyde-sulphuric acid at 620 nm are shown in Figure 2.

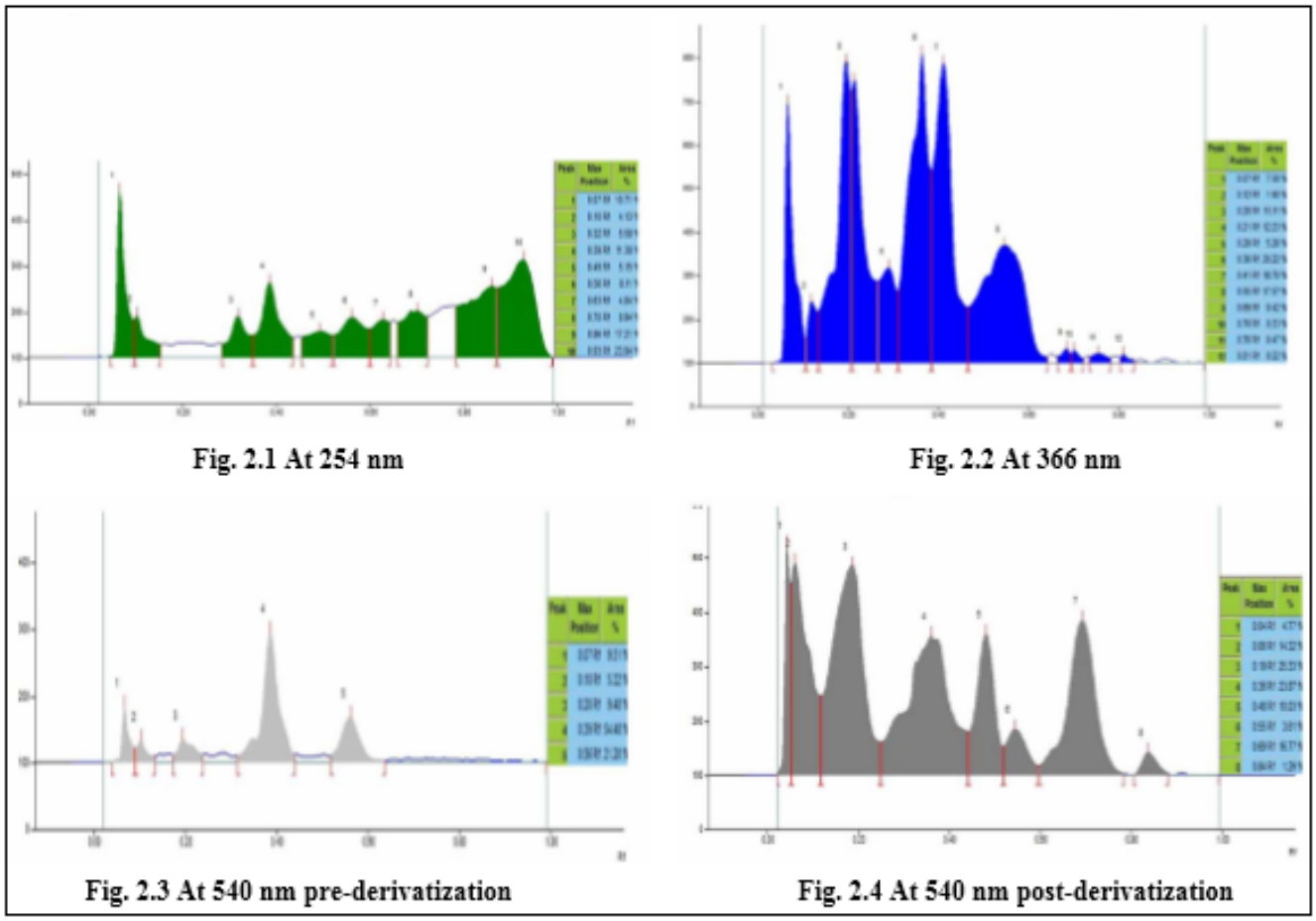

Fig 2: HPTLC Densitometric scan of n-hexane extract of *Cinnamomum sulphuratum*

The GLC chromatogram for the essential oil of *c. sulphuratum* is presented in Figure 3.

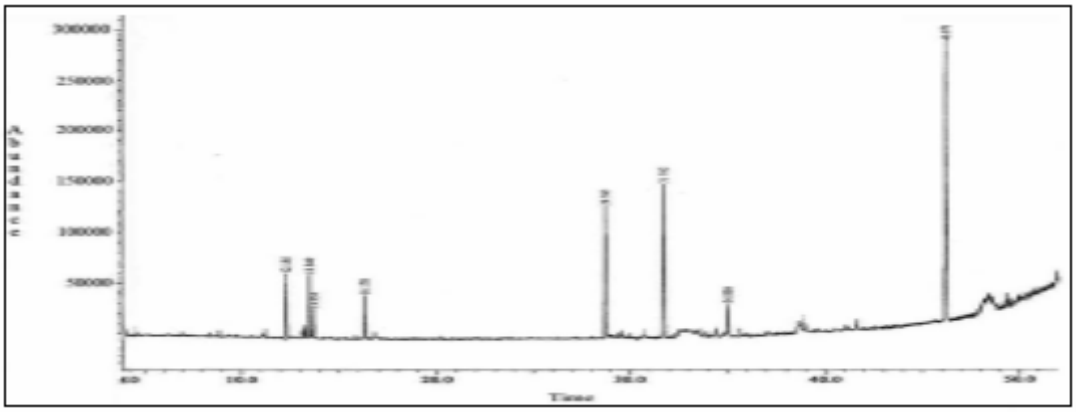

Fig 3: GLC chromatogram of the essential of *Cinnamomum sulphuratum*

Structure of the compounds detected by GC-MS is given in Figure 4.

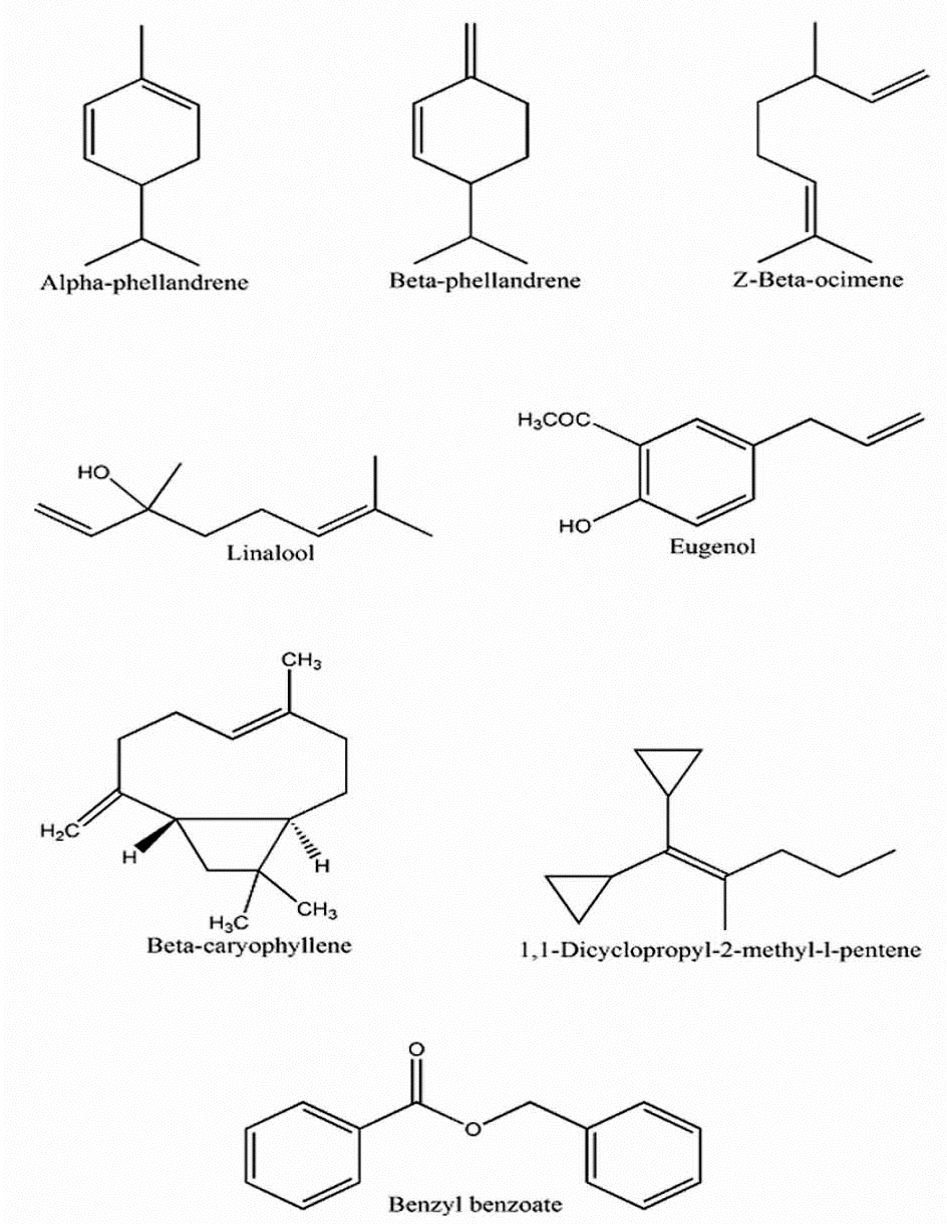

Fig 4: Structure of essential oil components from *Cinnamomum sulphuratum*

Compounds detected by the GC-MS of the essential oil *C. sulphuratum*, the respective RT values and the percentages of the compounds detected are tabulated in Table 3.

Table 3: Compounds detected by GC-MS of leaf essential oil from *Cinnamomum sulphuratum*

S No.	Compounds	RT	Area %	Baruah *et al*, 1999	Rameshkumar *et al*, 2006
1	*α*-phellandrene	12.155	6.32	+	-
2	*β*-phellandrene	13.349	6.64	-	-
3	Z-β-ocimene	13.555	2.53	-	+
4	Linalool	16.178	4.53	+	+
5	Eugenol	28.583	16.67	+	-
6	*β*-caryophyllene	31.582	20.63	+	+
7	1,1-dicyclopropyl-2-methyl-l-pentene	34.792	3.35	-	-
8	Benzyl benzoate	46.071	39.14	+	+

6. Discussion

It was felt worthy to investigate the chemical profile for establishing the identity of this species as it is a less known spice. In the present study, physico-chemical constants, HPTLC fingerprint and volatile oil composition by GCMS were recorded for the leaves of *C. sulphuratum* from Kodagu.

Results obtained for physico-chemical parameters such as loss on drying, pH of water soluble extractive, total ash, acid insoluble ash, *n*- hexane soluble extractive, alcohol soluble extractive, water soluble extractive and swelling factor can be used as diagnostic parameter for routine identification of *c. sulphuratum* leaves.

HPTLC fingerprinting revealed the presence of many phytoconstituents with their respective R_f values. The photo documentation of the plates showed numerous bands under UV 254, 366, white light, and after derivatisation with anisaldehyde-sulphuric acid reagent. On photo documentation under 254 nm there were 5 spots (Figure 1.1), 4 spots were detected under 366 nm (Figure 1.2), 3 spots under white light (Figure 1.3) and 7 spots after derivatisation with anisaldehyde-sulphuric acid (Figure 1.4, Table 2). Densitometric scan at 254 nm revealed 10 peaks corresponding to 10 compounds in the *n*-hexane extract in the solvent system used for the separation, compounds with R_f 0.07 (10.71%), 0.39 (11.38%), 0.86 (17.21%) and 0.93 (23.94%) were the major ones (Figure 2.1). Densitometric scan at 366 nm showed 12 peaks, compounds with R_f 0.20 (15.11%), 0.21 (12.23%), 0.36 (20.22%), 0.41 (18.70%) and 0.55 (17.57%) were the major peaks detected (Figure 2.2). Densitometric scan at 540 nm showed 5 peaks, compounds with R_f 0.39 (54.48%) and 0.56 (21.20%) were major ones (Figure 2.3). Densitometry at 620 nm showed 8 peaks, compounds with 0.19 (25.33%) and 0.36 (23.87%) were the major peaks detected (Figure 2.4).

On hydrodistillation the leaf yielded 0.65 % v/w of pale yellow fragrant essential oil which is lighter than water. GC-MS of the oil revealed 8 major volatile constituents (Figure 3 & 4 and Table 3). The oil was found rich in benzyl benzoate (39.14%) as reported by earlier workers [11]. *β*-Caryophyllene accounted for 20.63% followed by 16.67% of eugenol. Other constituents were *β*-phellandrene (6.64%), *α*-phellandrene (6.32%), linalool (4.53%), 1,1-dicyclopropyl-2-methyl-l-pentene (3.35%) and Z-β-ocimene (2.53%). *α* and *β*-phellandrene were not reported from *C. sulphuratum* growing in western Ghats by earlier workers; whereas *α*-phellandrene was reported in *C. sulphuratum* from northeast India by Baruah and Nath [8]. Out of 8 compounds identified, 3 compounds were reported from *C. sulphuratum* growing in south and north-east India; 5 compounds from *C. sulphuratum* growing in south India [11], and 4 compounds from *C. sulphuratum* growing in north-east India [8]. Presence of *β*-phellandrene and 1,1-dicyclopropyl-2-methyl-l-pentene is the first time report from the leaves of *C. sulphuratum* growing in Kodagu.

7. Conclusion

The chemical profile delineated would be helpful to differentiate *C. sulphuratum* from other related species

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