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Chemical analysis of leaf essential oil of *Cinnamomum verum* from Palni hills, Tamil Nadu

Anubhav Chakraborty*, Vishwam Sankaran, Murugan Ramar, David Raj Chellappan[1]
School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India
[1]Central Animal Facility, School of Chemical and Biotechnology,
SASTRA University, Thanjavur, Tamil Nadu, India
***Corresponding author:E-mail:sugindavid@yahoo.com; Phone: (+91) 9442076470**

ABSTRACT

Cinnamon (*Cinnamomum verum* J. Presl) leaf essential oil is well cited in literature for its phytochemical richness with eugenol being the primary constituent. However, the concentration of the phytochemical components varies depending on several factors like climatic and geographical conditions. Analyzing the essential oil obtained from *C. verum* leaves from Palni Hills, Tamil Nadu using GC-FID and GC-MS revealed 19 components, representing 97.6 % of the oil with eugenol (81.7%), linalool (3.8%) and benzyl benzoate (3.9%) as the main constituents. Further, the individual concentrations of these three major constituents were compared with the major constituents of leaf essential oil of *C. verum* from other geographical regions in India. Owing to the idea of volatile oils involving in protection and plant-plant communication, this comparative study relating variations in the oil composition due to agroclimatic and geographical conditions could aid in understanding plant diversities.

Keywords: *Cinnamomum verum*, essential oil, eugenol, linalool, benzyl benzoate

INTRODUCTION

Cinnamomum verum J. Presl (syn. *Cinnamomum zeylanicum* Blume) belonging to the Lauraceae family is a small evergreen tropical tree (growing up to 10 m high) native to Sri Lanka. In India, it is introduced and generally cultivated in farms and plantations in hilly regions of southern India. Cinnamon leaf and bark are used in the production of essential oils and also used as spices. The characteristic features of the leaves includes leathery appearance usually opposite, glabrous on both surfaces, leaf blade is greenish white abaxially, green and shiny adaxially, ovate or ovate-lanceolate (11 to 16 cm) with pointed tips (Li et al., 2008). Eugenol has been reported to be the main constituent in leaf essential oil (70-95%) (Senanayake et al., 1978). Other important constituents are linalool, cinnamaldehyde (Variyar and Bandopadhyaya, 1989), benzyl benzoate (Rao et al, 1988). Chemical composition of leaf essential oil of Literature well documents *C. verum* has been well documented (Mallavarapu et al., 1995, Raina et al., 2005, Kaul et al., 1996) and from the literature survey it could be seen that no work has been done on the chemical composition of cinnamon leaf oil from Palni Hills, Tamil Nadu which called for this investigation.

MATERIALS AND METHODS

Plant material: Leaves of *C. verum* were collected from the plants planted in a coffee plantation in Nallurkadu in Palni Hills (Kodaikanal), Tamil Nadu for extraction of essential oil. Herbarium voucher specimens (R. Murugan 86) were prepared for identification. The species was identified by Dr. R. Murugan, School of Chemical and Biotechnology, SASTRA University.

Extraction of essential oil: The leaf sample was dried in shade for about two weeks. The dried leaves were subjected to hydro-distillation in a Clevenger apparatus for about 5 h (Council of Europe, Pharmacopée Européenne, 1996). Colourless essential oil was obtained. A pinch of anhydrous sodium sulphate was added with oil sample to remove moisture content in the oil. The oil sample was stored at 4 °C until further analysis and studies.

Analyses of essential oil: Quantitative and qualitative analysis of essential oil were performed by Gas Chromatography- Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) respectively to determine the chemical components present in the essential oil and their respective percentage.

Agilent 7890B Gas Chromatograph equipped with Flame Ionization Detector (FID) was used to perform GC-FID analysis. A HP-5 capillary non-polar column (30 m length, 0.32 mm inner diameter and 0.25 µm film thickness) coated with 5% phenyl - 95% methyl polysiloxane was used. The initial oven temperature was kept 60 °C for 5 min. and then to 240 °C at the rate of 3 °C/min; injector and detector temperatures at 250 °C; Helium carrier gas at a linear velocity of 30 cm/sec and pressure at 93.6 kPa, flow rate of 1 ml/min; oil sample injection volume 1 µl and split ratio 1:10 was maintained. Quantitative data were obtained from FID area percentage without using internal correction factors.

PerkinElmer Clarus 500 Gas Chromatograph equipped with a Turbo Mass Gold Quadrupole Mass Spectrometer was used to perform GC-MS analysis. Here, Elite-5 capillary, non-polar column (30 m length, 0.25 mm inner diameter and 0.25 µm film thickness) coated with 5% phenyl - 95% dimethyl polysiloxane was used. The different conditions of operations included: An initial oven temperature 60 °C for 5 minute and raised to 240 °C at the rate of 3 °C/minute, final hold time 5 minute; Injector temperature at 270 °C; Helium carrier gas at the flow rate of 1 ml/minute; oil injection volume 1 µl dissolved in hexane; split ratio 1:20. Mass operating conditions were; Electron Ionization voltage 70 eV; Transfer line and ion source temperatures at 200 °C and 160 °C respectively; scan range 40-600 amu.

Retention index (RI) was determined relative to Natural hydrocarbons *n*-alkenes with carbon range from C_8 to C_{20}. (Kovats, 1958) The RI of the GC peaks were compared with those reported in literature and matching the mass spectra of the peaks with those of authentic compounds in literature (Adams, 2007) and database (NIST MS library 2005 - National Institute of Standards and Technology, USA) to identify the chemical constituents.The GC chromatogram of oil sample is showed in Figure 1.

RESULTS AND DISCUSSION

Leaves of *C. verum* upon hydro-distillation yielded 1.5% (v/w- on dry weight basis) colourless essential oil. The identified compositions are shown in Table 1. In total, 19 components amounting 97.6 % of the tested sample were determined. The major components present were eugenol (81.7%), linalool (3.8%) and benzyl benzoate (3.9%) and their biological activities as reported in literature are shown in Table 2.

On comparing these three constituents of the essential oil obtained from leaves of *C. verum* collected from different geographical locations, higher eugenol content was found to be in the plant from Bangalore (84.50%) (Mallavarapu et al., 1995) followed by Palni Hills (81.7%), Hyderabad (81.43%) (Mallavarapu et al., 1995), Little Andaman (76.60%) (Raina et al., 2005). The percentage composition of linalool was higher in the Little Andaman variety (8.50 %) (Raina et al., 2005) followed by Palni Hills (3.8%), Bangalore (3.70 %) and Hyderabad (1.57%) (Mallavarapu et al., 1995). The oil from Little Andaman variety lacked benzyl benzoate in its composition but was found to be higher in Hyderabad (8.29 %) (Mallavarapu et al., 1995) followed by Palni Hills variety (3.9 %) and in trace amount in Bangalore (0.13 %) (Mallavarapu et al., 1995). From the above facts it is conclusive that the essential oil from Palni Hills is best competitive with oils from the same plant species in other parts of Southern India.

On comparison with other studies it was found that these principle components do not change in their prevalence in the cinnamon oil despite changes in geographical distribution, season of procurement (Kaul et al., 1996) and other climatic influences. However, percentage composition of these components was found to vary across changing agroclimatic and geographical conditions. Evidence has been accumulating that the principle role of volatile oil in plant leaves can be categorized into two: protection and plant-plant communication (Bakkali et al., 2008).

In this context, the minor variation of some of the essential oil constituents across geographical regions and seasons might be due to the requirement for adopting versatility by the plants to adapt to changing needs for protection and communication. It still remains to be experimentally validated how changes in composition of these volatile oils across geography and climatic conditions affects plant-plant communication. Also to be experimentally validated is the role of each individual component in the context of communication between plants. It is possible that essential-oils could serve as a unique type of language involved in plant-plant communication with mild variations reflecting cultural diversity among plant species.

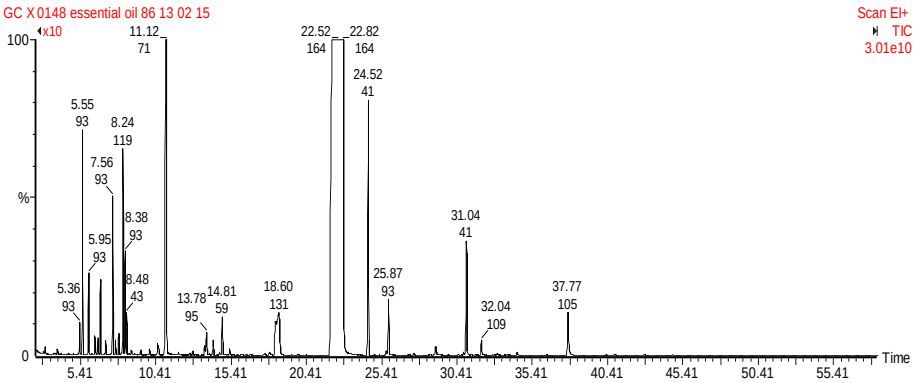


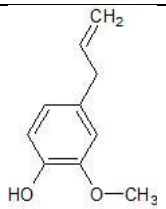
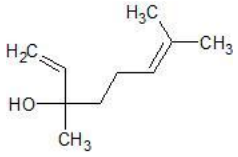
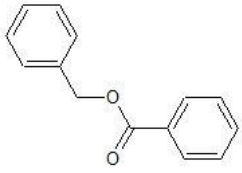
Figure.1.GC chromatogram of essential oil extracted from leaves of *C. verum*

Table.1.Constituents of the essential oil extracted from leaves of *C. verum*

SL. No	RI[a]	Name	Area %[b]	Method of identification
1.	933	α-Thujene	0.1	RI,MS
2.	936	α-Pinene	0.5	RI,MS
3.	952	Camphene	0.2	RI,MS
4.	981	β-Pinene	0.2	RI,MS
5.	1005	α-Phyllandrene	0.3	RI,MS
6.	1027	ρ-Cymene	0.4	RI,MS
7.	1031	β-Phellandrene	0.5	RI,MS
8.	1034	1,8-Cineole	0.1	RI,MS
9.	1100	Linalool	3.8	RI,MS
10.	1171	Borneol	0.1	RI,MS
11.	1192	α-Terpeneol	0.1	RI,MS
12.	1274	(*E*)-Cinnamaldehyde	0.8	RI,MS
13.	1363	Eugenol	81.7	RI,MS
14.	1422	(*E*)-Caryophyllene	1.7	RI,MS
15.	1440	γ-Elemene	1.1	RI,MS
16.	1455	(*E*)-Isoeugenol	0.3	RI,MS
17.	1579	Caryophyllene oxide	1.2	RI,MS
18.	1591	Humulene epoxide II	0.6	RI,MS
19.	1764	Benzyl benzoate	3.9	RI,MS
		Total identified	**97.6**	

[a] Retention indices as tested on HP-5 column using the homologous series of C_{8-20} n-alkanes
[b] Relative percentages of components based on GC-FID peak areas

Table.2.Biological activities of major phytochemical constituent of *C. verum* leaves essential oil

Major phytochemical constituent of *C. verum* leaves essential oil	Molecular formula and structure*	**Biological Activities
Eugenol	 $C_{10}H_{12}O_2$	Anesthetic, anti-inflammatory, antioxidant, antispasmodic, antiulcer, vasodilator
Linalool	 $C_{10}H_{18}O$	Analgesic, anesthetic, antispasmodic, anti-inflammatory, hypnotic, sedative, anti-allergic
Benzyl benzoate	 $C_{14}H_{12}O_2$	Antiasthamatic, antispasmodic, antitumour, hypotensive, myrorelaxant

*** Structures drawn using ChemSketch, C10E41, 2015**
**** Dr. Duke's phytochemical and ethnobotanical databases. ARS/USDA, 1994.**

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