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Chemical analysis of leaf essential oil of *Cinnamomum tamala* from Arunachal Pradesh, India

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ABSTRACT

The leaf of *Cinnamomum tamala* T. Nees and Ebrum has been widely used in culinary preparation for its rich aroma and medicinal benefits. The presence of volatile aromatic essential oil rich in eugenol is well reported in the leaves of *C. tamala*, making the plant commercially important for perfumeries. The essential oil extracted from C. tamala, however, varies in phytochemical constituents and concentration of its components with changing geographical and climatic conditions. GC-FID and GC-MS analysis of leaf essential oil from *C. tamala* specimen obtained from East Siang District, Arunachal Pradesh, revealed eugenol (60.2%), α-Phellandrene (11.7%) and β-Phellandrene (7.2%), α-Pinene (2.8%), Elixene (1.8%), *cis*-Caryophyllene (1.6%), Myrcene (1.5%) and Limonene (1.4%) as its major constituents. Findings indicate that literature published on essential oils, henceforth, should provide more detailed description of environmental conditions during plant procurement in order for literature to shed more light on essential oils and their role in plant intelligence.

Keywords: *Cinnamomumtamala*, essential oil, eugenol, alpha-phellandrene, beta-phellandrene

INTRODUCTION

Cinnamomum tamala T. Nees and Ebrum.is a small evergreen tropical tree of the Lauraceae family. The species is native to India, Nepal, Bhutan and China and is widely distributed in the northern parts of India. It is commonly known as *Tejpat* in the northern parts of India and the leaf of the plant (*Tejpat* leaf) is widely used in culinary preparations for its aroma and also employed in folk medicine (Rema, 2005). Cinnamon leaf and bark are widely used in the production of essential oils and also used as spices. The leaves have a leathery appearance and arranged in an alternating manner (sometimes sub-opposite in young branchlets). The leaves are glabrous on both surfaces; leaf blade is greenish white and opaque, abaxially green and shiny adaxially. The midrib extends to leaf apex, basal lateral veins very elevated abaxially, slightly elevated adaxially, transverse veins undulate and veinlets are reticulate (Flora of China, 2008). Though wide literature exists on the essential oil obtained from the leaves of *C.tamala*, it's very little accounts for correlating the physiological significance of differing essential oil components with geographical changes of plant procurement.

MATERIALS AND METHODS

Plant material: *C. tamala* leaves were obtained from the plants in Pasighat, East Siang District, Arunachal Pradesh for extraction of essential oil. Herbarium voucher specimens (*Jeyaprakash*86) were prepared for identification and the species was identified by Dr. R. Murugan, School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu.

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

Analyses of essential oil: Quantitative and qualitative analyses 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.

GC-FID analysis: Agilent 7890B Gas Chromatograph equipped with Flame Ionization Detector was used to perform GC-FID analysis. The setup included 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.

Chromatography Conditions: A temperature of 60 °C was maintained for 5 min. The oven temperature was then altered to 240 °C at the rate of 3 °C/min; the temperatures of the injector and detector were maintained at 250 °C; The carrier gas used was Helium and was passed at linear velocity- 30 cm/sec with a pressure of 93.6 kPa. Flow rate of carrier gas was 1 ml/min; Sample injection volume was set as 1 μl. Split ratio was maintained at 1:10. FID area percentage was calculated to obtain quantitative data. This was done without using internal correction factors.

GC-MS analysis: Instrument used included a Perkin Elmer Clarus 500 Gas Chromatograph. The analyzing component of the chromatogram was a Turbo Mass Gold Quadrupole Mass Spectrometer. Chromatograph was made of an Elite-5 capillary with a non-polar column of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness. The column was coated with 5% phenyl - 95% dimethyl polysiloxane. Operation conditions: An initial oven temperature 60 °C for 5 min. This was raised to 240 °C at the rate of 3 °C/min. The final hold time was set as 5 min.; Sample was injected at 270 °C with Helium as carrier gas. Carrier gas flow rate was set as 1 ml/min and sample injection volume was 1 µl (dissolved in Hexane); A split ratio of 1:20 was set.

Operating conditions of Mass Spectroscope: Electron Ionization voltage of 70 eV, The scan range was between 40-600 amu. Ion source temperature was maintained 160 °C, The Transfer Line was kept at 200 °C.

Natural hydrocarbons(*n*-alkenes with carbon range from C_8 to C_{20}) was used as standard to calculate Retention Index (RI)(Kovats, 1958).Chemical constituents of the oil was determined by comparing RI obtained from GC peaks with RI reported in literature and by matching the mass spectra of the peaks with the spectra of authentic compounds in literature (Adams, 2007) and database (NIST MS library 2005 - National Institute of Standards and Technology, USA).

RESULTS AND DISCUSSION

Hydro-distillation of leaves of *C. tamala* yielded 1.2% (v/w- dry weight basis) colourless essential oil. Table 1 shows the identified compositions owing in total of 37 components that amounts to 98.6 % of the tested sample. The major components of the oil were found to be Eugenol (60.2%), α-Phellandrene (11.7%), β-Phellandrene (7.2%), α-Pinene (2.8%), Elixene (1.8%), *cis*-Caryophyllene (1.6%), Myrcene (1.5%) and Limonene (1.4%).

The percentage of the primary constituent, Eugenol (60.2%) obtained from leaves of *C. tamala* procured from Pasighat, Arunanchal Pradesh was compared with the essential oil obtained from leaves of *C. tamala* of various other geographical locations. Higher Eugenol content was found to be in the plant variety of Loklaubung (91.4%) followed by Mizoram (91.1%) followed by Namching (86.8%), Manipur (77.8%), Assam (77.4%), Molnom (77%), Meghalaya (74.3%), Nungshai (74%), Sagolband (63.8%) (Rana, 2012), Pasighat (60.2%) and Renkai (41.8%) (Rana, 2012).

Reports from other regions include Kangra District in Himachal Pradesh (11.57%) and Drang Forest, Himachal Pradesh (9.98%) and Pakistan (0.10%) (Rema, 2005) Another major deviation was found in case of leaf essential oil from Southern part of India which had Eugenol composition of only 0.078%. On the contrary Cinnamaldehyde (44.9%) and Trans-cinnamyl acetate (25.33%) composition was found to be higher in this region (Kumar,2012). A variation in composition of the oil can be seen and this can be attributed to environmental changes. The composition of other components can also be seen to change across regions. Of the 13 regions considered for this study eugenol was present in 92% of the cases. Other major components were found to have prevalence as follows: eugenyl acetate in 70% of the cases, α-Phellandrene in 54% of cases, Bicyclogermacene also in 54% of cases (Rana, 2012). Though the percentage composition of these components in the oil varies between regions, the prevalence of some components was found to be high. However, it must also be noted that none of the components reported as major constituents in the North Indian varieties was found as a major constituent in the oil from the plant variety of southern Indian (Kumar, 2012). This could be attributed to an adaption of the plant to different environments.

The principle role of volatile oil in plant leaves are protection and plant communication (Bakkali, Averbeck, 2008). Clearly, essential-oils serve as a means for plants in adapting to environmental stress. It remains to be seen if this behavior of adapting to conditions using essential oils is solely determined by the genotype of the plants, or if such behavior is an outcome of intelligent adaptation by plants to changing environmental conditions. Thus it remains to be studied in this context if a variety of *C. tamala* that grows in one region would produce oil of same characteristics if planted in a different geographical region. To gain more insights on this however, plant procurement details in literature must be made more elaborate with information on prevalent biotic and abiotic factors pertaining to the site and time of procurement. The physiological benefits of the principle components also need to be well established to make stronger correlations between environment and essential oil based intelligent adaptation in plants. In addition, eugenol is extensively cited in literature (Dr. Duke's phytochemical and ethnobotanical databases, 1994) for its anesthetic, anti-inflammatory, antioxidant, antispasmodic, antiulcer, vasodilating properties and being the primary component in the *C. tamala* leaves essential oil, a study investigating the physiological action of the oil might render outstanding results.

CONCLUSION

The GC-MS analyses revealed that the essential oils are mainly composed of 37 components. The percentage of the primary constituent obtained from leaves of *C. tamala* procured from Pasighat, Arunanchal Pradesh was found to be Eugenol (60.2%).

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