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Original Article

Volatile profiling from heartwood of East Indian sandalwood tree

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ARTICLE INFO

Article history:

Received 9 April 2013

Accepted 25 April 2013

Available online 16 May 2013

Keywords:

Santalum album

GC–MS

Heartwood

Santalol

Volatile

ABSTRACT

Background: Volatile aroma compounds are important characteristics determining essential oil quality. The heartwood of sandalwood tree, *Santalum album* L. deposits the sandalwood oil with enormous therapeutic potentials. The majority of the biological activities are attributed to the sesquiterpenoid alcohols i.e. santalols and hence, there is the need to explore the presence of other volatile bioactive constituents from heartwood.

Methods: We used a gas chromatography–mass spectrometry (GC–MS) method employing an ion trap quadrupole (ITQ) mass analyzer to identify and quantify volatiles from solvent extracted heartwood oil of the East Indian sandalwood tree, *S. album* L.

Results: A total of 46 constituents composed of a great variety of n-alkanes, sesquiterpenoids, fatty acids, aldehydes, naphthalene derivatives, methyl esters, alcohols, carotenoid degradation products, and acetates were identified. Oxygenated sesquiterpenoids, i.e., Z- α -santalol and epi- β -santalol and sesquiterpene hydrocarbons were identified as the major constituents in the extracted oil.

Conclusion: Results indicate that GC–ITQ–MS is a robust tool for qualitative and quantitative analysis of volatile constituents of the heartwood of sandalwood tree. Furthermore, the constituents reported may lead to the discovery of novel phytopharmaceuticals from sandalwood tree.

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1. Introduction

The East Indian sandalwood tree, *Santalum album* L. (a Santalaceae member) is a woody, tropical tree acclaimed for costliest heartwood and the essential oil obtained from it. Upon steam-distillation the heartwood yields precious sandalwood oil that has over 90% santalols (α - and β -santalols and their sesquiterpenoid isomers).¹ The sesquiterpenoid rich sandalwood essential oil is accumulated beyond 15 years of growth of the

tree. The yield ranges from 2.5 to 6% depending on the age of the tree, the color of the heartwood, individual tree under-study, sampling site within the tree and the environment of growth.² Reported sandalwood essential oil constituents are sesquiterpenoids,³ triterpenoids and phenylpropanoids.⁴ The major essential oil components are ‘santalane-backbone bearing’ sesquiterpenoids as santalenes and santalols.^{1,3,5,6} However, in sandalwood oil α -santalol is more abundant (46%) than β -santalol (20%)^{7–9} although both differ in their

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<http://dx.doi.org/10.1016/j.jopr.2013.04.030>

stereochemistry and biological activity. However, reported literature on total volatile constituents of this tropical essential oil-yielding tree is scanty. Besides, it is highly likely that the non-sesquiterpenoid constituents, other than santalols could play critical roles in several ethnopharmacological and therapeutic properties. The GC–MS profiles of commercially available sandalwood oil obtained by the process of steam-distillation constitute one of the first reports in this direction.¹ Previously conducted investigations on heartwood volatiles of sandalwood tree focused mostly on santalol biosynthetic pathway intermediates.⁶ In lieu of the available limited information on the wood volatiles, in this study, we investigated the solvent extractable volatiles from the matured heartwood by GC–MS.

2. Methods

2.1. Plant material and extraction of volatiles

The heartwood of a 15-year-old tree grown in the Department of Biotechnology, Indian Institute of Technology Kharagpur campus, was bored at 100 cm height from the ground and chips/powders were collected and air dried for 48 h. Solvent extraction was done in eluotropic series (n-pentane, n-hexane, chloroform and diethyl ether) in 500 ml volume Erlenmeyer flasks, for 12 h each, at 25 ± 5 °C, with intermittent shaking in a 10% (w/v) ratio of plant materials to solvent. During extraction 0.01% (w/v) BHT (butylated hydroxytoluene) was added as a synthetic antioxidant to protect the phytochemicals from auto oxidation and served as an internal standard. Obtained extracts were dried over Na_2SO_4 , pooled and were concentrated *in vacuo*, in a rotary evaporator (N–N Series, Eyela, Tokyo) at 40 °C. The volatile yield was determined by gravimetric method and was expressed as percentage of starting plant material. The extracts were reconstituted in n-hexane and proceeded for GC–MS analysis.

2.2. Gas chromatography–mass spectrometry analysis

The pooled volatile fraction was analyzed by GC–MS using a Thermo Trace GC Ultra™ gas chromatograph system, equipped with a 30 m (l) \times 0.25 mm (*i.d.*), 0.25 μm film thickness, non-polar TR-5MS fused silica capillary column, connected to an ion trap quadrupole (ITQ) mass selective detector, with a unit mass resolution. The split was 1:50, with helium as the carrier gas at a flow rate of 1 ml/min, while the damping gas flow was 0.3 ml/min. The initial oven temperature was set to 40 °C for 1 min. The GC oven temperature program was as follows: 40 °C–220 °C, by ramping at 3 °C, and held at 220 °C for 20 min. The injector temperature was maintained at 220 °C and the transfer line was held at 220 °C. The detection was performed by a Thermo ITQ 900™ mass spectrometer in the EI mode (ionization energy of 70 eV, ion source temperature of 180 °C, emission current of 220 μA). The acquisition was made in full scanning mode (mass range 50–900 m/z ; 3 scans/s). Maximum ionization time was 25 ms. A solvent delay time of 5 min (set off) was used to avoid overloading the mass spectrometer with hexane. Data collection, analysis and integration were performed using the software XCalibur™ (version 2.0.7). Areas

were recorded under all detectable peaks, and percent composition was calculated by taking area of peak divided by total chromatogram area \times 100. The volatile oil yield was determined by gravimetric means and calculated as percentage of starting fresh weight heartwood. For identification of constituents, mass spectra were compared with data from the National Institute of Standards and Technology (NIST, Washington DC, USA) and Dr. Duke's Phytochemical and Ethnobotanical Database (<http://www.ars-grin.gov/duke/>).

2.3. Statistical analyses

Statistical analysis was performed with SPSS software package (version 17) (SPSS Inc., Chicago, IL, USA). To understand the difference in values of parameters obtained from assays, one-way analysis of variance (ANOVA) was performed. Data provided were obtained from four inter-day runs of the GC–MS.

3. Results and discussion

The volatile yield obtained from chipped heartwood was 0.045%, *i.e.*, 45 mg g^{-1} dry weight. This yield is comparable to those obtained from transition zone and central core of heartwood tissue *i.e.* 30–90 mg g^{-1} dry weight heartwood as reported.⁶ The results show that the extracted fraction is a complex mixture of 46 identified constituents which represented about 93.4% of the total volatile yield (Table 1). The dominant sesquiterpenoids in the volatile fraction were *Z*- α -santalol and *epi*- β -santalol, whereas the following constituents have been reported in sandalwood oil¹⁰ *i.e.*, compounds – 20, 22, 25, 34, 36 and 38. Sesquiterpenoids were traced from their characteristic mass fragments of m/z 161 and m/z 204. To the best of our knowledge the occurrence of the following sesquiterpenoid compounds are reported for the first time from Indian sandalwood tree, *i.e.*, compounds 18, 23, 24, 27, 29, 30 and 32 (Table 1). Other lesser known sesquiterpenoids in sandalwood tree that have been identified include, germacrene A, bicyclogermacrene, and β -elemene. We report for the first time, the occurrence of following compounds from heartwood volatiles, *i.e.*, 1, 3, 5–7, 10–16, 21, 31, 33, 37, 39–46; in total 30 known compounds from literature. The hemiterpene, 2-methyl butanoic is derived from 3, 3-dimethylallyl pyrophosphate and isopentenyl pyrophosphate, and has the highest odor impact among the non-sulfurous odorants.¹¹ The co-occurrence of β -caryophyllene and caryophyllene oxide, suggests oxidation of β -caryophyllene into the latter. The constituent α -ylangene, a tricyclic sesquiterpene is responsible for the 'pepper' aroma of the heartwood derivatives. 2-octen-1-al is derived from autoxidation of unsaturated fatty acids.¹² The aldehyde, 5-methyl-2-furfural is a sugar degradation product, along with benzaldehyde possibly, contribute to the powerful sweet and spicy odor of sandalwood oil. Furthermore, the saturated and unsaturated volatile C_6 and C_9 compounds are mainly responsible for the "fresh green" odor of the leaves. *Cis*-3-hexenyl acetate is derived via lipooxygenase cleavage of fatty acids within seconds of injury¹³ are one of the "green-leaf volatiles" with a grassy odor that are typically found in the case of damaged leaves. The

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