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SCREENING OF ACTIVE PHYTOCHEMICAL COMPOUNDS IN *SANTALUM ALBUM* L. THROUGH GC-MS

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ABSTRACT

Sandalwood is a valuable tree associated with immense medicinal and natural fragrance with commercial significance. The sandal is known for its oil which is produced from the wood of sandal on distillation. The oil content of the different wood samples in *Santalum album* is varied *viz.,* heartwood (7.79%), rootwood (8.12%) and sapwood (1.26%).The root wood reaches highest oil content compared to heartwood and sapwood. The study shows that, 14 active phytocompounds viz., P-Benzoquinone (0.17%, 0.14%), á-Santalene (0.86%, 1.21%) TereSantalol (1.17%, 1.19), Epi- â-Santalene (1.02%, 1.28%), â-Santalene (1.87%, 1.89%), 2-Carene (0.36%, 0.42%), á-Curcumene (0.41%, 0.40%), á-Santalol (54.28%, 55.16%), Z-á-trans-Bergamotol (2.06%, 2.15%), E-Cis,epi-â-Santalol(7.26%, 6.23%), â-Santalol(27.01%, 27.40%), E-Nuciferol(1.63%, 0.83%), trans â Santalol (1.02%, 0.64%) and cis Lanceol (0.89%, 1.08%) were identified in the heartwood and rootwood extract and 11 active phytocom pounds were identified in the sapwood, But only 6 active phytocompounds are similar to heartwood and rootwood extract of *Santalum album* by GC-MS study. Present investigation conclude that the quality and value of sandalwood oil is commercially determined by the level of ásantalol and â santalol present in the part of the wood.

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INTRODUCTION

Sandal (*Santalum album* L.) is a commercially and culturally important plant species belonging the family santalaceae (Manoj kumar Reddy and Sukanya Subramanian, 1998). Sandal is considered is as one of the most valuable tree in the world. The sandal is known for its oil which is pronounced as the most famous East Indian sandal wood oil which is produced from the heartwood of sandal on distillation (Bachi and SindhuVeerendra, 1991*)*. The sandal wood oil has been known in the perfume industries for several centuries (Srinivasan *et al*. 1992). Besides perfume industry the sandal oil and powder obtained from sandal wood found multifarious utility in cultural and religious festivals coupled with the utility in wide range of medicinal use particularly in Asian Countries (Subasinghe 2013). Photochemical constituents are the basic sources for the establishment of several pharmaceutical industries the constituents present in the plant which play a significant role in the identification of crude drugs (Pradeep Kumar et al. 2011). Phytochemicals screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, phenols, saponins, steroids, tannins, terpenoids, flavonoids etc. In the search for phytochemicals that may be of benefit to the pharmaceutical industry (Sreedevi and Damodharam, 2015).

The medicinal values of the most of the plants are not yet evaluated scientifically for assessment of their potential as useful drugs. Destruction of forest and lack of proper documentation have made some of these species rare, threatened or endangered. Developing of modern biotechnology based conservation methodology like tissue culture, germ plasm culture and screening of plant metabolites for discovering of potential drugs of pharmaceutical industry, can adopted for scientific exploration of these valuable bioresourses (DebajitKalita et al, 2012). This drives the need to screen Sandalwood for novel bioactive compounds as plant based drugs are biodegradable and safe. A natural product plays an important role in the field of new drugs research and development because of their low toxicity, easy availability and cost effective (Tariq and Reyaz, 2013).

S.album produces the best fragrant material, and is one of the oldest perfumery materials and the powdered heartwood, upon distillation, yields East Indian Sandalwood Oil. The oil is highly rated for its fixative properties and for its persistent, heavy, sweet and woody odour because of the Phytochemicals constituents (Arun Kumar *et al.,* 2012). Present investigation was focused on the identification, quantification and characterization of the phytochemicals constituents from different wood samples viz., heartwood, root wood and sapwood samples using GC-MS analyses and to show the oil content of the above indicated wood samples.

MATERIALS AND METHODS

Plant materials

The wood samples of *Santalum album* were collected from the dead trees of arboretum in Forest College & Research Institute, Mettupalayam and Tamil Nadu.

Extraction of essential oil

The oil was extracted from 3 different parts of the wood samples viz., Heart wood, Root wood, Sap wood. All the 3 types of wood samples were crashed and powdered individually. 10g of air dried powdered wood samples was treated with liquid nitrogen and then extracted with dichloro methane for three days in soxhlet apparatus. After remotion of the solvent under reduced pressure, the extract was distilled with odour- free water for 2 hours to obtain 350ml of distillate. The distillate was saturated with NaCl and extracted with fresh distilled diethyl ether (3x100ml). The ether solution was dried under Na_2SO_4 and concentrated through rotary evaporator to obtain yellow oil used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis (Bhuiyan et al., 2009). The resulting oil was weighed and expressed as percentage of the weight of wood powdered used for extraction. The oils were then stored separately in sealed container under refrigeration prior of bio-chemical analysis.

GC-MS Analysis

The extracted Sandal wood oil from the three wood samples was analyzed by Gas Chromatography- Mass Spectrometry (GC-MS). Phyco-constituents were detected using a Hewlett Packard 5890 Series II gas chromatographic system (Hewlett Packard, Waldbronn, Germany) equipped with HP-5971 mass selective detector (MSD, Hewlett Packard, Palo Alto, CA, USA) and capillary column (30m×0.25mm×0.25mm) was used with helium at a 1 ml min^{-1} as a carrier gas, GC oven temperature was kept at 110°C for two minutes and programmed to 280°C at the rate of 5°C min^{-1} and kept constant at 280°C for10 minutes. The split ratio was adjusted to 1:20 and injection volume was 2 ìl. The injection and detector temperature was 250°C. The GC-MS electron ionization mode was 70 eV. Mass range was from m/z 45-450 amu.

For the gas chromatographic-mass spectrometric analysis of the volatile compounds a 30 Mts, ID: 0.25 mm, FILM: 0.25ìm DB-5MS fused-silica capillary columns (Agilent, USA) were used at a helium flow rate of 1 ml/min. The oven temperature was kept at 80^{0}C for 1 min and raised to 300^{0}C for 5min. The identification of compounds was obtained by comparison with Wiley 275 Mass Spectra Library (Manilal, 2010; Surendra Singh BishtandHemanthraj, 2014).

Oils were diluted to 1% with diethyl ether prior to analysis. Different constituents separated were identified by parallel comparison of their retention times and mass Spectra with NIST, PEST and PESTIC mass spectral database.

RESULTS AND DISCUSSION

Fourteen Compounds were identified in oil isolated from heartwood oil and Rootwood oil and eleven compounds were identified in the Sapwood oil extracted from the identified harvested sandal wood. Significance difference among the oil samples was observed.

The Composition of different constituents was expressed as peak area percent. There are 14 major compounds which are identified in the 3 different samples *viz.*, á-Sautalene, â-Sautalene, á-Sautalol, â-Sautalol. Table 2 shows that á-Sautalol is the highest proportion in all the three types of Sandal wood followed by â-Sautalol were also found to be higher comparing to other compounds. Based on the different samples of sandal wood, Root wood yields higher in á-Sautalol and â-Sautalol followed by heart wood and very minimum amount of á-Sautalol and â-Sautalol present in the Sapwood.

The compounds were identified in Heartwood oil and

Table 1: Comparison of sandal wood oil percentage obtained by different part of wood samples

Wood Sample	Oil (%) Experiment 1	Experiment 2	Experiment 3	Average
Heart Wood	7.81	7.71	7.86	7.79
Root Wood	8.36	7.95	8.06	8.12
Sap Wood	1.19	1.33	1.26	1.26

Table 2: Phytochemical compounds in the oil isolated from different wood extract of *Santalum album*

S.No	Compounds	Mol. Formula	Reuteutiontime time	Percent composition Heart Wood	Root Wood	Sap Wood
1.	p-Benzoquinone	$C_6H_4O_2$	10.21	0.17	0.14	-
2.	á-Santalene	$C_{15}H_{24}$	26.66	0.86	1.21	0.16
3.	TereSautatol	$C_{10}H_{16}O$	30.01	1.17	1.19	0.29
4.	Epi- â-Sautalene	$C_{40}H_{56}$	30.64	1.02	1.28	-
5.	â-Santalene	$C_{15}H_{24}$	30.96	1.87	1.89	0.11
6.	ë-Carene	$C_{10}H_{16}$	31.15	0.36	0.42	-
7.	á-Curcumene	$C_{15}H_{24}$	31.56	0.41	0.40	-
8.	á-Santalol	$C_{15}H_{24}O$	37.68	54.28	55.16	7.63
9.	Z- á-trans-Bergamotol	$C_{15}H_{24}O$	37.99	2.06	2.15	-
10.	E-cis, epi- â -Santalol	$C_{15}H_{24}O$	38.31	7.26	6.23	0.46
11.	â-Santalol	$C_{15}H_{24}O$	38.86	27.01	27.40	1.96
12.	E-Nuciferol	$C_{15}H_{22}O$	38.91	1.63	0.83	-
13.	Trans - â - Santalol	$C_{15}H_{24}O$	39.36	1.02	0.64	-
14.	Cislanceol	$C_{15}H_{24}O$	39.99	0.89	1.08	-

Rootwood oil is relatively similar *viz*., P-Benzoquinone (0.17%, 0.14%), á-Santalene (0.86%, 1.21%) TereSantalol (1.17%, 1.19), Epi- â-Santalene (1.02%, 1.28%), â-Santalene (1.87%, 1.89%), 2 - Carene (0.36%, 0.42%), á-Curcumene (0.41%, 0.40%), á-Santalol (54.28%, 55.16%), Z-á-trans-Bergamotol (2.06%, 2.15%), E-Cis,epi-â-Santalol (7.26%, 6.23%), â-Santalol (27.01%, 27.40%), E-Nuciferol(1.63%, 0.83%), trans â Santalol (1.02%, 0.64%) and cisLanceol (0.89%, 1.08%), heartwood and rootwood respectively (Table 2). But the Sapwood oil contain only 6 Compounds are similar heartwood and rootwood samples *viz*., á-Santalene (0.16%), TereSantalol (0.29%), â-Santalene (0.11%), á-Santalol (7.63%), E-Cis, epi-â Santalol (0.46%) and â Santalol (1.96%) (Table 2). The Sapwood oil of Sandal wood includes some other compounds include Hydroquinone, á-Bergamotene, (Z)-â-Farnesene, â-Bisabolol also present. But this compound is not present in Heartwood and Rootwood. Similar result found in the *Aquilaria* species of different grades of oil isolated from infected and non-infected wood (Jayachandran *et al.,*2014). Previous reports indicate that, there are quantitative and compositional differences in oils obtained from young and mature sandalwood trees and across heartwood sampled at different levels in the tree (Shankara narayana and Parthasarathi, 1987). It is also noteworthy, that, yields of secondary metabolites depends on the intrinsic characteristics of plant material, environmental, and genetic aspects, or by extrinsic aspects (Muzika *et al.,* 2006).

Previous studies showed almost the relatively same percentages in the case of major constituents were á-santalol (33.55-35.32%), â-santalol (17.16-18.96%), epi-â-santalol (2.23-3.51%), epi-â-santalene (0.80-1.69%), á-santalene (0.56-1.6%),â-santalene (1.12-2.35%), and á-bergamotol (4.03-7.77%), But different, much higher percentages in the case of different part of wood in the *santalum album*. That proves the great influence of the climate conditions on the chemical composition of the essential oils, even if we consider the same species which was harvested from the same place, but in different consecutive years. It is possible that, during the vegetative stage, depending on the temperature, different metabolic reactions might take place. Moreover the biosynthesis of sandalwood oil sesquiterpenes along with heartwood content of wood core and its composition (Surendra Singh Bisht, 2014).

Chemical composition of crude extracts from infected woods of Aquilariamalaccensis were compared to that of healthy wood and commercial agarwood. Agarwood substances were extracted in methanol and were subjected to GC-MS analyses. The major compounds were chromone derivative, aromatic compounds, sesquiterpenes, monoterpenes, sterols and fatty acid methyl ester. Aromatic compounds constituted of aldehyde, phenol, ether and ketone groups. In the agarwood extract of the juvenile fungal-elicited tree but not in the healthy wood (Jong *et al.,* 2014).

Similar studies found in the species viz,. *Adhatodavasica* (Manoj Kumar, *et al.,* 2014), *Sargasamwitti* and *Gracilariaedilus* (Hebsibah, 2010*), Swertiachirayita* (Manoj kumar, 2015), *Costus specious, Gloriossasu perba* and *Rauvolfia serpentine* (Rakesh dadsena *et al.,* 2013), *Sebastiania chamaelea* (Shanthi sree *et al.,* 2010)

Phytochemical research has led to the isolation and characterization of an impressive number of biologically active chemical constituents from sandalwood and its oil. Yet, possibilities are there for finding novel chemical entities. Pharmacological investigation have not only validated the traditional medicinal practices but also discovered several new therapeutic potential of sandalwood and its constituents (Rakesh Kumar *et al.,* 2015).

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