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Metabolite profiling in heartwood of farm-grown *Pterocarpus santalinus* using GC-MS

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Abstract

Pterocarpus santalinus Linn is popularly known as Red Sanders. The heartwood of Red Sanders fetch high price for its utility and colour. Santalin is a natural red dye obtained from the heartwood of Red Sanders, which is used as a colouring agent in pharmaceutical preparations and foodstuffs. The heartwood of *Pterocarpus santalinus* is known for its pharmaceutical uses. Heartwood ethanol extract of farm-grown *Pterocarpus santalinus* revealed the presence of different groups of alkaloids, phenol compounds, benzenes, esters, glycerides, triterpenoids, diterpenoids, steroids and aldehydes. The major compounds identified in terms of peak area are Ethyl iso-allocholate, 4-Cyano-6-dicyanomethyl-2-methyl-2,5-diphenyl-1,2-dihydropyridine, 2-Naphthalenemethanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene-, [2R-(2 $\alpha,4\alpha,8\alpha$)]-, β -Bisabolol, 6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-o, (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol, Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, $\alpha,\alpha,6,8$ -tetramethyl-, stereoisomer, 13-Oxadispiro[5.0.5.1]tridecane, 2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl, 1-Heptatriacotanol, 1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene, trans-Z- β -Bisabolene epoxide, 4,22-Stigmastadiene-3-one, Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-2-yl ester and ζ -Sitostenone. The above components are present in the heartwood of farm grown Red Sanders and are known for its antimicrobial, antibacterial, antioxidant, anti-inflammatory and anticancer activities.

Keywords: Farm-grown red sanders, heartwood, GC-MS metabolite profiling, biological activities

1. Introduction

Pterocarpus santalinus L.F, (Red Sanders) is one of the highly valuable timber in the world. This tree is an endemic species belongs to the family Fabaceae (Vedavathy; 2004) [15]. It is distribution to naturally confined to few parts of South India in Andhra Pradesh and Tamil Nadu (Durai, 2021) [5]. The dye extracted from the heartwood is Santalin and is used in pharmaceutical preparations, colouring, foodstuffs, high-class alcoholic liquors and textiles. It is also used to prepare pesticides, explosives, plastics and drugs (Cai *et al.*, 2004) [1]. Preparation of heartwood extract have been widely documented in traditional Indian ayurvedic medicine (Gupta and Uniyal, 2003) [6].

Phytochemical analysis of *Pterocarpus santalinus* revealed the presence of flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, tannins and glycosides and specific phytoconstituents such as β -sitosterol, lupeol, epicatechin, lignans and pterostilbenes (Pullaiah *et al.*, 2019) [13]. Highly specific metabolites present in Red Sanders are pterocarpol, pterocartriol, santalin A, B and C, isopterocarpalone, pterocarpodiolones, β -eudesmol and cryptomeridiol (Yoganasimhan *et al.*, 2000) [16]. The heartwood is known to have antihyperglycaemic, antipyretic, anti-inflammatory, anthelmintic, aphrodisiac and diaphoretic properties are used (Soundararajan *et al.*, 2016) [14]. Fourteen major chemical compounds were identified in the ethanolic extract of *Pterocarpus santalinus* heartwood from natural forest (Karthick and Parthiban, 2019) [8]. The present study was carried out to assess the metabolite profile in heartwood used of farm-grown Red Sanders.

2. Materials and Methods

2.1 Preparation of the sample

The heartwood samples of farm-grown Red Sanders were collected from wind-fallen and damaged trees. The heartwood samples were chipped into 20 mm size using a chipper and pulverized in Wiley mill. Ten grams of crushed heartwood powder after defatted with

petroleum ether was fed in Soxhlet extractor for 6 hours with ethanol to extract the phytochemicals (Karthick and Parthiban, 2019; Mohammed *et al.*, 2016) ^[8, 11].

2.2 Gas chromatography and mass spectrometer (GC-MS) analysis

The metabolite profile of its heartwood extract from farm-grown Red Sanders was analysed by using Gas Chromatography and Mass Spectrometer (Perkin Elmer Clarus SQ8C) using DB-5, MS capillary non-polar column (30m, ID; 0.25mm and film thickness of 0.25µm). One microlitre of ethanolic extract was utilised as the sample size, and 1ml/min of helium was employed as the carrier gas during analysis. The oven temperature range was set at 285 °C to 80 °C (80 °C for 5min, 4 °C rate 260 °C and 2 °C rate 285 °C hold for 10 minutes). The MS scanning range was set to 45-650Da. The MS additionally included an internal pre-filter to

lessen neutral particles. The data system features two built-in libraries, NISTA and WILEY 9, which together contain more than one million references, for searching and matching the spectrum. The National Institute of Standards and Technology (NIST4) database and WILEY 9 libraries were used to analyse the mass spectrum from the GC-MS. The relative percentage of extract constituents were expressed with peak area normalization.

3. Results and Discussion

Metabolite profiling of heartwood samples of farm-grown Red Sanders was detected by GC-MS in ethanolic extract. A total of 40 chemical compounds were identified in the ethanolic extract of farm-grown Red Sanders heartwood sample and is presented in Table 1 and the chromatograph is depicted in Figure 1.

Table 1: Metabolite profile of heartwood ethanolic extract of farm-grown Red Sanders detected by GC-MS

S. No.	Retention time (minutes)	Compound name	Peak Area (%)
1.	3.023	Ethyl iso-allocholate	2.016
2.	3.429	4-Cyano-6-dicyanomethyl--2-methyl-2,5-diphenyl-1,2-dihydropyridine	1.741
3.	3.709	Benzeneethanamine, 2-fluoro-á,3,4-trihydroxy-N-isopropyl-	0.525
4.	3.844	[2-(5-Hydroxypent-2-ynyl)-3-oxocyclopentyl]thioacetic acid, S-t-butyl ester	0.267
5.	4.064	à-N-Normethadol	0.365
6.	4.214	1,7-Octanediol, 3,7-dimethyl	0.504
7.	15.678	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-à,à,4a,8- tetramethyl-, (2R-cis)-	0.567
8.	15.803	3-O-Methyl-d-glucose	0.600
9.	16.109	2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]-	4.216
10.	16.744	à-Bisabolol	0.950
11.	17.339	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-o	0.879
12.	18.634	7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	0.267
13.	18.820	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	0.275
14.	19.435	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	0.881
15.	20.175	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.584
16.	20.350	5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1- methylethylidene)-, (8S-cis)	0.275
17.	20.410	Longipinocarveol, trans	0.603
18.	20.770	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, à,à,6,8-tetramethyl-, stereoisomer	27.205
19.	20.925	13-Oxadispiro[5.0.5.1]tridecane	1.481
20.	21.180	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	1.987
21.	21.396	Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-	0.411
22.	21.666	(4aS,7R)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyl-4,4a,5,6,7,8- hexahydronaphthalen-2(3H)-one	0.299
23.	22.016	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	0.858
24.	22.201	1-Heptatriacotanol	1.285
25.	22.381	1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	4.516
26.	22.511	trans-Z-à-Bisabolene epoxide	2.847
27.	22.851	4,22-Stigmastadiene-3-one	0.721
28.	22.911	Pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3á,5à,6à,16à)-	0.302
29.	23.436	4,22-Stigmastadiene-3-one	1.239
30.	23.686	Ethyl iso-allocholate	2.218
31.	23.882	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4- (hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8amethanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR- (1aà,2à,5á,5aá,8aà,9à,10aà)]-	0.358
32.	24.042	Ethyl iso-allocholate	0.446
33.	24.267	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	0.504
34.	24.592	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-2-yl ester	0.839
35.	27.603	Ethyl iso-allocholate	0.464
36.	28.238	Butanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4- (hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo-1H-2,8amethanocyclopenta[a]cyclopropa[e]cyclodecen-5-yl ester, [1aR- (1aà,2à,5á,5aá,8aà,9à,10aà)]-	0.410
37.	28.383	à-Sitosterol	0.413
38.	28.433	2-Methylaminomethyl-5-nitrobenzophenone	0.545
39.	28.883	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3- [(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 5, 9,9a-triacetate, [1aR-(1aà,1bá,4aá,5á,7aà,7bà,8à,9á,9aà)]-	0.267
40.	29.489	ç-Sitostenone	17.570

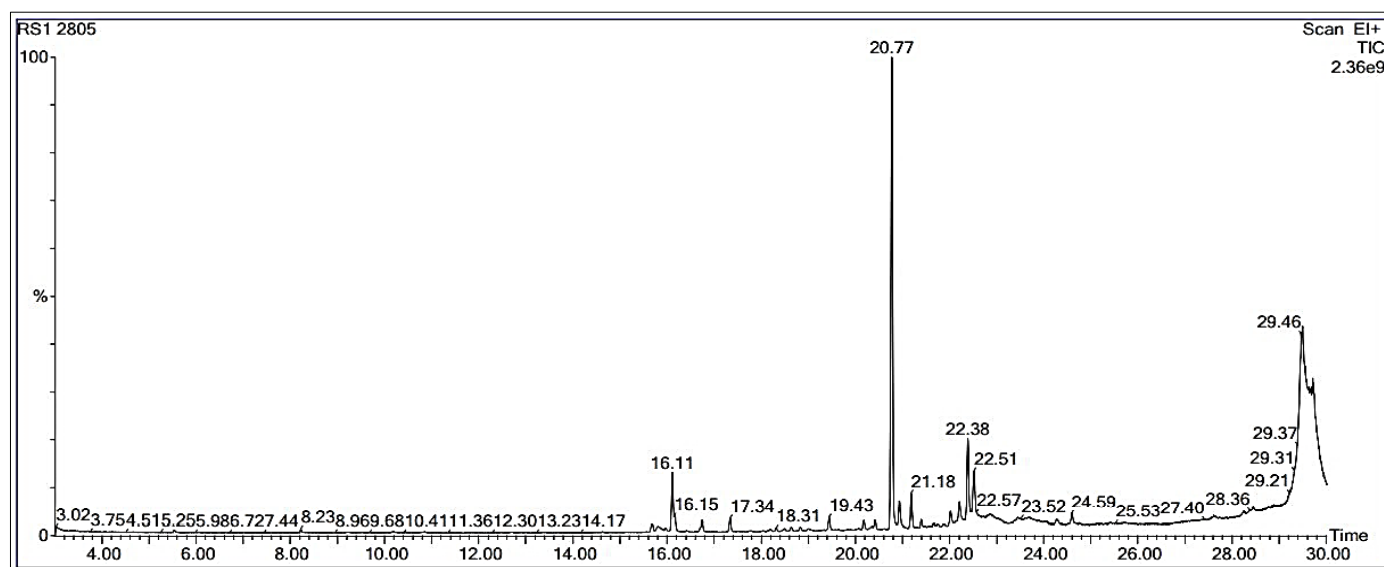


Fig 1: Chromatograph of heartwood ethanol extract of farm-grown *Pterocarpus santalinus*

Among the 40 chemical compounds, 16 major compounds present in the heartwood of farm-grown Red Sanders in terms of % peak area are viz., Ethyl iso-allocholate (5.14%), 4-Cyano-6-dicyanomethyl-2-methyl-2,5-diphenyl-1,2-dihydropyridine (1.74%), 2-Naphthalenemethanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene-, [2R-(2 $\alpha,4a\alpha,8a\alpha$)]- (4.78%), α -Bisabolol (0.95%), 6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-o (0.88%), (1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol (0.88%), Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol (5.14%), $\alpha,\alpha,6,8$ -

tetramethyl- (27.21%), stereoisomer (5.14%), 13-Oxadispiro[5.0.5.1]tridecane (1.48%), 2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)- (2.71%), Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl (1.36%), 1-Heptatriacotanol (1.29%), 1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene (4.51%), trans-Z- α -Bisabolene epoxide (2.84%), 4,22-Stigmastadiene-3-one (2.05%), Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-2-yl ester (0.84%) and ζ -Sitostenone (17.57%) (Table 2).

Table 2: Biological activities of the major chemical compounds present in farm-grown Red Sanders heartwood ethanol extract detected by GC-MS

Peak No	RT	Area %	Compound Name	Molecular Formula	Molecular Weight g/mol	Secondary Metabolite	Reported Activity
1.	3.023	2.016	Ethyl iso-allocholate	C ₂₇ H ₄₈ O ₅	452.7	steroid	Antioxidant, Anti-inflammatory, Antiarthritic, Antimicrobial
2.	3.429	1.741	4-Cyano-6-dicyanomethyl-2-methyl-2,5-diphenyl-1,2-dihydropyridine	C ₇ H ₆ N ₂ O	134.14	amine	Phosphodiesterase inhibitor
3.	16.109	4.216	2-Naphthalenemethanol, decahydro- $\alpha,\alpha,4a$ -trimethyl-8-methylene-, [2R-(2 $\alpha,4a\alpha,8a\alpha$)]-	C ₁₅ H ₂₆ O	222.37	sesqui-terpenoid	Anti-inhibitory, Antibacterial and Antioxidant properties
4.	16.744	0.950	α -Bisabolol	C ₁₅ H ₂₆ O	222.37	Sesqui-terpene	Cosmetics and skincare products primarily as a fragrance
5.	17.339	0.879	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-o	C ₁₅ H ₂₄ O	220.35	Sesqui-terpenoid	Antibacterial and Antioxidant properties
6.	19.435	0.881	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	C ₁₅ H ₂₈ O ₂	240.38	Sesqui-terpenoid	Antibacterial and Antioxidant properties
7.	20.770	27.205	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, $\alpha,\alpha,6,8$ -tetramethyl-, stereoisomer	C ₁₅ H ₂₄ O	220.35	Sesqui-terpenoid	Anti-oxidant and Anti-inflammatory
8.	20.925	1.481	13-Oxadispiro[5.0.5.1]tridecane	C ₁₂ H ₂₀ O	180.29	volatile organic compound	Fragrance and flavouring agent
9.	21.180	1.987	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	C ₁₂ H ₂₀ O	180.29	Alcohol	Food and Color Additives
10.	22.016	0.858	Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl	C ₁₅ H ₂₆ O ₃	254.36	Diterpene	Anti-inflammatory activity

11.	22.201	1.285	1-Heptatriacotanol	C ₃₇ H ₇₆ O	537.0	Alcoholic compound	Antimicrobial, Antioxidant, anti-inflammatory.
12.	22.381	4.516	1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene	C ₁₃ H ₂₂	178.31	Terpenoid	Antibacterial, Antioxidant and cosmetics
13.	22.511	2.847	trans-Z-à-Bisabolene epoxide	C ₁₅ H ₂₄ O	220.35	Sesqui-terpenoid	Antimicrobial, Anti-inflammatory, and Antioxidant properties
14.	23.436	1.239	4,22-Stigmastadiene-3-one	C ₂₉ H ₄₆ O	410.7	Steroids	Antibacterial
15.	23.686	2.218	Ethyl iso-allochololate	C ₂₇ H ₄₈ O ₅	452.7	Steroid	Antioxidant, Anti-inflammatory, Anti-arthritis, Antimicrobial
16.	24.592	0.839	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8, 8a-octahydronaphthalen-2-yl ester	C ₁₇ H ₂₆ O ₃	278.4	Miscellaneous phytochemicals	Antibacterial properties
17.	29.489	17.570	ç-Sitostenone	C ₂₉ H ₄₈ O	412.7	Steroids	Cosmetics

The Gas Chromatography Mass Spectrometer analysis of farm-grown Red Sanders heartwood revealed the presence of different groups of metabolites like alkaloids, benzenes, esters, phenol, glycerides, triterpenoids, diterpenoids, steroids and aldehydes. 2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]-found in *Populus nigra* is used as an anti-inhibitory agent against microbes (Dongli *et al.*, 2014) ^[4]. à Bisabolol is an essential oil extracted from *Matricaria chamomilla* and *Populus balsamifera* (Kiarash *et al.*, 2015) ^[9]. Tricyclo [4.4.0.0(2,7)] dec-8-ene-3-methanol, à,à,6,8-tetramethyl-, stereoisomer found in *Pterocarpus marsupium* is known for its anti-oxidant and anti-inflammatory activities (Maruthupandian and Mohan, 2014). 13-Oxadispiro [5.0.5.1] tridecane is a volatile compound and is used as a fragrance compound (Pa *et al.*, 2008). Perhydrocyclopropa[e]azulene-4,5,6-triol, 1,1,4,6-tetramethyl found in *Eucalyptus citriodora* is known for its insecticidal activity (Nebras *et al.*, 2016) ^[12]. 1-Heptatriacotanol found in *Nigella sativa* is used as an antimicrobial, antioxidant and anti-inflammatory agent (Hameed *et al.*, 2016) ^[11]. 1,3,3-Trimethyl-2-(2-methyl-cyclopropyl)-cyclohexene is a volatile compound found in essential oil of *Jatropha ribifolia* and is found to possess antibacterial and antioxidant activity and is also used in cosmetics (Silva *et al.*, 2013) ^[2]. ç-Sitostenone found in *Michelia compressa* is used in cosmetics (Chung-Yi Chen *et al.*, 2015) ^[3]. Ethyl iso-allochololate from a medicinal rice Karungkavuni inhibits dihydropteroate synthase in *Escherichia coli* and is used for its antioxidant, anti-inflammatory, anti-arthritis and antimicrobial activity (Sudha *et al.*, 2016) ^[7].

4. Conclusion

In the present study, the ethanolic extract of farm-grown *Pterocarpus santalinus* heartwood is rich in bioactive compounds. Most of the compounds in this fraction possess antibacterial, antioxidant, anti-inflammatory and antimicrobial properties. The heartwood of farm-grown Red Sanders comprising of rich source of phytochemicals and will open up new avenues in terms of value addition of Red Sanders in future and fetch more returns to tree growers particularly Red Sanders.

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