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Determining composition of volatiles in *Couroupita guianensis* Aubl. through headspace-solid phase micro-extraction (HS-SPME)

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ABSTRACT

Composition of volatile components in *Couroupita guianensis* Aubl. flowers was analyzed using headspace-solid phase micro-extraction (HS-SPME), followed by capillary gas chromatography and mass spectrometry (GC-MS) separation and identification. In all, 75 compounds were identified accounting for 96.32% of the total volatiles present. The major groups of compounds present were oxygenated terpenoids (35.66%), alcohols (26.92%), esters (17.36%), mono-and sesqui-terpenoids (8.64%), aldehydes and ketones (4.71%), hydrocarbons (1.68%), phenols (0.18%), acids (0.754%) and heterocyclic compounds (0.42%) constituted a small proportion of the volatile profile. The most abundant individual constituent was eugenol (18.95%) followed by nerol (13.49%), (E,E) farnesol (12.88%), (E,E)-farnesyl acetate (6.68%), trans ocimene (6.02%), nootkatone (4.64%), geraniol (2.94%), 2-isopropenyl-5-methyl-4-hexenyl acetate (2.69%), cedr-8-en-13-ol (2.58%), (E,Z)-farnesyl acetate (2.40%) and methyl (11E)-11-hexadecenoate (2.041%). Analytical comparison of composition of volatiles in the flowers, obtained by different methods of extraction, viz., solvent extraction, micro-simultaneous extraction and headspace-solid phase micro-extraction, revealed specific variations in relative concentrations of the constituent chemicals. Linalool was the major chemical (21.5% and 14.9%) in solvent extract and micro-simultaneous extract, respectively, but appeared in negligible quantity (0.16%) in head-space analysis.

Key words: *Couroupita guianensis*, volatiles, headspace-solid phase micro-extraction (HS-SPME), capillary gas chromatography and mass spectrometry (GC/MS)

INTRODUCTION

Couroupita guianensis Aubl, or the Cannonball tree, has always been a botanical curiosity due to the unique shape of its flowers and fruits. The plant, belonging to the family Lecythidaceae, is native to the tropics of the northern part of South America and to the West Indies (Heywood and Chant, 1982). In India, the tree is grown in the vicinity of Shiva temples, as, Hindus revere it as sacred, it being known as 'Shivalingam' in Hindi. It is a fast growing, evergreen tree attaining a height of up to 30m. The fragrant, orange-red flowers are borne on long, thick, tangled extrusions from the trunk. Fruits are spherical, brown and large as a cannon ball. Besides its ornamental value, the tree has several medicinal properties. Infusion from the flowers is used for treating colds and stomach ache (Anon., 1950) and the bark is used for treating hypertension, tumors and inflammations (Stanz *et al*, 2009). In Brazil, its leaves are widely used as an analgesic (Mariana *et al*, 2010) and for treating skin diseases (Satyavati *et al*, 1976). The flowers emit a strong, sweet, spicy fragrance. Previous efforts on chemical examination revealed presence of linalool, eugenol, nerol, tryptanthrin, farnesol, indigo, indirubin, isatin, linoleic acid, α, β- amirins, carotenoids, sterols and some acidic and phenolic compounds (Sen *et al*, 1974; Bergman *et al*, 1985; Wong and Tie, 1995; Rane *et al*, 2001; Rajamanickam *et al*, 2009). Wong and Tie (1995) identified 41 compounds responsible for fragrance in *Couroupita* flowers using solvent extraction, of which eugenol, linalool, (E,E)-farnesol and nerol were the major ones. Similar results were obtained by Andrade *et al* (2000) from fresh flowers using the micro-simultaneous extraction method. Variation in relative concentrations of the major fragrance components occurs due to a difference in the method of extraction employed. The objective of the present study was to identify aroma compounds that most likely represent the fragrance of *Couroupita guianensis* flowers, using the headspace-solid phase micro-extraction (HS-SPME) technique. HS–SPME is now a well-established and very popular technique for head-space (HS) sampling in several fields, including study

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of composition of HS volatiles in medicinal and aromatic plants, flowers and fruits where it has assumed an ever-increasing importance. HS-SPME is an easy and non-destructive method of extraction of volatiles, therefore, a more accurate method than others. Solvent extraction and simultaneous micro-extraction method could modify the compounds due to the destructive way of sample preparation besides the high temperatures used for extraction. Studies on head-space extraction and analysis of flower volatiles (Flamini *et al*, 2003; Deng *et al*, 2004 and Belliardo *et al*., 2006) report direct sampling using SPME to avoid interferences from non-volatile matrix components (Pawliszyn, 1997).

MATERIAL AND METHODS

Plant material

Fresh, fully opened *Couroupita guianensis* flowers were collected in the morning during the month of May, 2013 from full-grown plants located near the garden of ICAR-Indian Institute of Horticultural Research, Bengaluru. Volatile fragrance constituents were extracted by headspace-solid phase micro-extraction (HS-SPME) technique and analyzed using GC–MS/MS.

SPME extraction of volatiles

A manual SPME holder and three commercial SPME fibers (procured from Supelco Inc. Bellefonte, PA, USA) were used in the study. SPME fibers were conditioned in a GC injector port as recommended by the manufacturer, at a temperature of 250°C for 3hrs before use in volatile extraction. SPME fiber types DVB/CAR/PDMS (Divinylbenzene/Carboxen/polydimethylsiloxane), 50/30 µm, highly crossed-linked (Supelco Inc., Bellefonte, PA, USA) were used for extraction of head-space volatile compounds from flowers.

Extraction process used for head-space volatiles was as per Flamini *et al* (2003) and Deng *et al (*2004). Soon after plucking, six *Couroupita* flowers were transferred to each of the two 250ml conical flasks (with screw caps and silicon rubber septum) and capped immediately. The samples were kept at room temperature (25 ±1°C) for 10-15 minutes to accelerate transfer of analytes for reaching equilibration in the head-space. After the equilibration-time was up, sampling was done by inserting pre-conditioned SPME fiber into the head-space of the flask for 1 hour at room temperature (25 ±1°C).

GC analysis

After extraction of head-space volatiles, the SPME device was inserted into the injector port for gas chromatographic analysis, and was held in the inlet for 10 minutes for desorption. GC-FID analysis was done using Varian-3800 Gas Chromatograph, equipped with FID detector. Nitrogen (1ml/min) was used as a carrier gas. The components were separated on VF-5, capillary column from Varian, USA, 30m x 0.25mm i.d., 0.25µm film thickness. The injector temperature was set at 260°C and all injections were made in split mode (1:5). The detector temperature was maintained at 270°C and the temperature programme used for the column was as follows: 50°C for 5 min, followed by an increment of 4°C/min till 170°C, held for 2 min; subsequently, increased by 5°C/min till it reached 250°C and, then, a constant temperature of 250°C was maintained for 7 minutes. The total run-time was 60 minutes.

GC/MS analysis

GC/MS analysis was carried out in the system consisting of a Varian-3800 Gas Chromatograph coupled to a Varian-4000 Ion-Trap mass spectra detector. The ion trap, transfer line and ion source temperatures were maintained at 190°C, 240°C and 200°C, respectively. A fused-silica capillary column VF-5ms from Varian, USA, with 30m x 0.25mm id, 0.25mm film thickness was used for the analysis. Helium was used as carrier gas with flow rate of 1ml/min. The mass spectrometer was operated in the external electron ionization mode of 70eV, with full mass scan-range 45–450amu. Temperature programmes used for the column were the same as described for GC-FID analysis.

Total volatile production was estimated by a sum of all GC-FID peak areas in the chromatogram and individual compounds were quantified as relative per cent area. Individual volatile compounds were identified by comparing their retention index (RI) which was determined using homologous series of n-alkanes (C_5 to C_{32}, procured from Sigma-Aldrich) as Standard (Kovats, 1965) and comparing mass spectra with the available two spectral libraries, using Wiley and NIST-2007.

RESULTS AND DISCUSSION

GC and GC-MS separation and identification of volatile components of *Couroupita* flowers extracted by headspace-solid phase micro-extraction (HS-SPME) resulted in identification of 75 compounds (Table 1). The total percentage of compounds identified was 96.32%, in

Table 1. Volatile components of *Couroupita guianensis* flowers estimated using headspace-solid phase micro-extraction (HS-SPME) method

Name of the compound/group	Retention Index	Area (%)
Hydrocarbons		
1. 1,3,5,5-Tetramethyl-1,3-cyclohexadiene	1028	0.058
2. 2-Methyl-2-bornene	1045	0.132
3. Eicosane	2011	0.569
4. Heneicosane	2103	0.707
5. Triecosane	2298	0.217
Total		1.684
Monoterpenoids		
6. β-Pinene	974	0.143
7. 3-Carene	1010	0.112
8. γ-Terpinene	1018	0.112
9. β-Phellandrene	1027	0.215
10. Limonene	1033	0.122
11. cis-Ocimene	1039	0.781
12. trans-Ocimene	1052	6.021
13. α-Terpinene	1057	0.096
14. Terpinolene	1075	0.112
15. Mentha-1,3,8-triene	1111	0.098
16. allo-Ocimene	1127	0.086
Total		7.899
Sesquiterpenoids		
17. α-Bergamotene	1445	0.095
18. β-Caryophyllene	1455	0.108
19. Germacrene D	1468	0.102
20. (Z,E)-α-Farnesene	1491	0.138
21. (E,E)α-Farnesene	1504	0.195
22. Bicyclogermacrene	1528	0.098
Total		0.736
Oxygeneted terpenoids		
23. Linalool	1095	0.164
24. 6-Camphenol	1118	0.112
25. cis-Verbenol	1131	0.665
26. 2-Pinen-4-ol	1146	0.095
27. cis-Limonene oxide	1148	0.103
28. Z-Thujanol	1165	0.055
29. (-)-Borneol	1173	0.121
30. Myrtenol	1192	0.132
31. Nerol	1223	13.489
32. Isogeraniol	1232	1.128
33. Geraniol	1258	2.942
34. Geranial	1268	1.178
35. Nerolidol	1568	0.153
36. Caryophyllene oxide	1585	0.112
37. (2Z,6E)-Farnesol	1682	0.266
38. (Z,Z)-Farnesol	1715	0.924
39. (E,E)-Farnesol	1725	12.881
40. (E,Z)-Farnesol	1742	1.072
41. Longifolenaldehyde	1876	0.065
Total		35.657
Phenolics		
42. Carvacrol	1304	0.096
43. 2,3,5,6-Tetramethylphenol	1321	0.088
Total		0.184

Table 1. Contd.

Name of the compound/group	Retention Index	Area (%)
Alcohols		
44. (E)-6-Nonen-1-ol	1124	0.095
45. (5-Isopropyl-2-methyl-1-cyclopenten-1-yl) methanol	1199	0.064
46. α-Methyl-benzeneethanol	1208	1.490
47. 2-(2,2,4-Trimethyl-3-cyclopenten-1-yl) ethanol	1233	0.385
48. Eugenol	1358	18.952
49. Methyleugenol	1392	0.112
50. Dihydro-β-ionol	1405	0.054
51. (E)-Isoeugenol	1463	0.059
52. Cedrenol	1603	0.079
53. Cedr-8-en-13-ol	1672	2.576
54. Z-9-Pentadecenol	1749	1.077
55. Z-11-Pentadecenol	1772	0.403
56. (6E,10E)-3,7,11,15-Tetramethyl-1,6,10, 14-hexadecatetraen-3-ol	2049	1.577
Total		26.923
Acids		
57. Myristic acid	1765	0.652
58. Pentadecanoic acid	1821	0.102
Total		0.754
Aldehydes and Ketones		
59. Isopulegone	1155	0.068
60. Nootkatone	1845	4.637
Total		4.705
Esters		
61. Methyl salicylate	1193	0.209
62. Z-Methyl geranate	1298	0.470
63. Citronellyl acetate	1348	0.122
64. 2-Isopropenyl-5-methyl-4-hexenyl acetate	1375	2.693
65. (Z,Z)-Farnesyl acetate	1810	0.507
66. (E,E)-Farnesyl acetate	1818	6.682
67. (E,Z)-Farnesyl acetate	1838	2.396
68. Methyl (11E)-11-hexadecenoate	1883	2.041
69. Methyl (9Z)-9-hexadecenoate	1892	1.221
70. (3Z)-3-Hexenyl benzoate	1568	0.121
71. Hexyl benzoate	1577	0.132
72. Ethyl (9E)-9-hexadecenoate	1969	0.762
Total		17.356
Heterocyclic compounds		
73. 2-Methylfuran	603	0.210
74. Indole	1289	0.156
75. (E)-3-(4,8-dimethyl-3,7-nonadienyl)-furan	1552	0.053
Total		0.419

which the major groups of compounds were: oxygenated terpenoids (35.66%), alcohols (26.92%), esters (17.36%), mono-and sesqui-terpenoids (8.64%) and aldehydes and ketones (4.71%) (Fig. 1). Hydrocarbons (1.68%), phenols (0.18%), acids (0.754%) and heterocyclic compounds (0.42%) constituted a small proportion of the volatile profile.

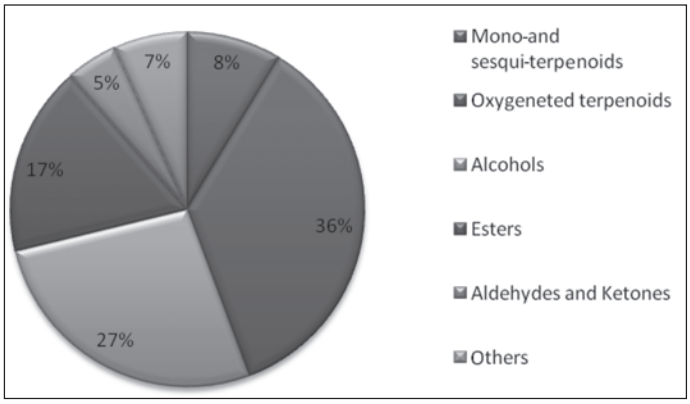

Fig 1. Relative abundance of various groups of volatile compounds in *Couroupita guianensis* flowers

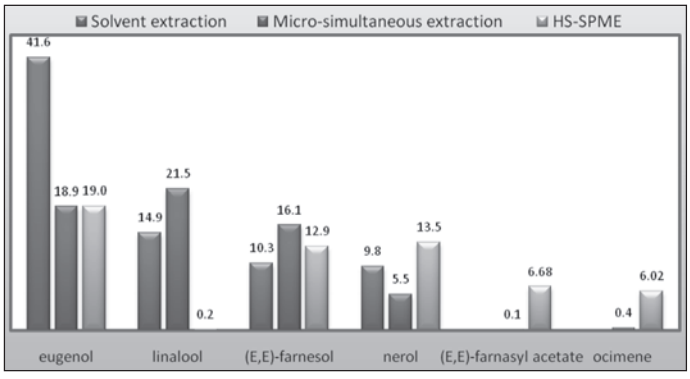

Fig 2. Variation in percentage of major chemical compounds in *Couroupita guianensis* flower fragrance obtained by solvent extraction, micro-simultaneous extraction and head-space solid phase micro-extraction (HS-SPME) techniques

The most abundant individual constituent was eugenol (18.95%), followed by nerol (13.49%), (E,E)-farnesol (12.88%), (E,E)-farnesyl acetate (6.68%), trans-ocimene (6.02%), nootkatone (4.64%), geraniol (2.94%), 2-isopropenyl-5-methyl-4-hexenyl acetate (2.69%), cerd-8-en-13-ol (2.58%), (E, Z)-farnesyl acetate (2.40%) and methyl (11E)-11-hexadecenoate (2.041%).

Comparison of volatile composition of *Couroupita guianensis* flowers obtained in the present study with earlier published methods of solvent extraction (Wong and Tie, 1995) and micro-simultaneous extraction (Andrade *et al*, 2000) revealed some variations in relative concentrations of the constituent chemicals (Fig. 2). Earlier studies reported linalool as a major constituent imparting aroma to orange-flower (21.5% and 14.9%), respectively in the volatiles profile. However, it appeared in negligible quantity (0.16%) in head-space analysis, where citrus aroma is attributed to higher percentage of nerol (13.49%). Eugenol, which is responsible for strong spicy nutmeg or clove-type odor of the flower, registered high percentage (18.9%) in both micro-simultaneous extraction and HS-SPME method, but comparatively lower than in the solvent extraction method (41.6%). Head space analysis also recorded higher percentage of (E,E)-farnesol (12.88%) and (E,E)-farnesyl acetate (6.68%) among the volatiles. These compounds add an oily floral note to fragrance-profile. Presence of ocimine, similarly, was observed only in HS-SPME method, and was reported to be negligible when estimated by the other methods. The variation in relative concentrations of major fragrance components observed in earlier studies could be due to different methods of sample preparation. In the earlier studies, 37 to 41 flavour compounds were identified whereas, in the present study, 75 compounds were identified covering 96.35% of all the compounds present. Therefore, HS-SPME method in our study was found to be better than solvent extraction and simultaneous micro-extraction methods of volatile extraction.

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