Journal of Medicinal and Aromatic Plant Sciences 36(1-2) (2014) 8-15

Solid phase micro extraction and GC-MS analysis of headspace volatiles of seed and cake of *Pongamia pinnata* (L.) Pierre

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Article History

Received: 24th June, 2013
Revised: 26th October, 2013
Accepted: 26th October, 2013

Key words

Bio-pesticide
Gas chromatography-mass spectrometry
Headspace volatiles
Pongamia pinnata

ABSTRACT

The composition of seed and cake volatiles of Pongamia pinnata (L) Pierre extracted by solid phase micro extraction was analyzed by GC and GC-MS. A total of 84 and 81 volatile compounds were identified from the headspace samples of seed and cake, respectively. The volatiles belonged to ten major groups of compounds comprising hydrocarbons (aliphatic and aromatic), terpenoids, alcohols, phenols, aldehydes and ketones, acids, esters, oxo compounds, sulphur compounds and nitrogen compounds. The most abundant individual compound present in the volatile fraction of both seed and cake was 2-chloroacetophenone (19.58% and 19.47%, respectively). Significant differences in the proportion of sulphur compounds, phenols and nitrogenous compounds between seed and cake were observed while there were little differences in others. The study found several compounds in the volatile fractions of both seed and cake reported to have an adverse effect on pests and pathogens of crops. This finding provides the first experimental evidence for the use of Pongamia volatiles as a bio-pesticide for crop protection.

INTRODUCTION

Pongamia pinnata (L) Pierre, also called *Derris indica* (Lam.) Bennet and *Pongamia glabra* Vent, is one of the few nitrogen fixing trees producing seeds that are known to have insecticidal and nematicidal activities [8]. The tree, commonly known as Indian beach tree or Karanja, is native to tropical and temperate Asia including parts of India, China, Japan and Malaysia. *Pongamia*, a fast growing tree species of Family leguminaceae, is found in almost all parts of India as an avenue tree. The seeds of *Pongamia* have been used as insecticide for crops in India from time immemorial [2] and is reported to contain, on an average 28–34% oil with a high percentage of polyunsaturated fatty acids [19]. The *Pongamia* seed oil, known as *honge* oil, is a valuable product known to possess strong insecticidal properties [8]. The prinicipal furanoflavonoids present in the seed oil are karanjin, pongamol, pongapin, glabrin, karanja chromene, karanjone and pongaglabrone [10, 14, 20]. Studies on ethanolic and methanolic extracts of *Pongamia* seeds have demonstrated the oviposition deterrent activity [22] and the potential for further development into a botanical insecticide against *Helicoverpa armigera* (Hubner) under field conditions [17]. The

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de-oiled seed cake containing flavonoids, uranoflavonoids, and furan derivatives are known to possess pesticidal activity and is also used as fertilizer in organic production practices. The work on insecticidal potential of karanj has been reviewed [13] and is found to be mainly restricted to its oil components.

In a field experiment conducted using *Pongamia* seed and cake, it was accidentally discovered that tomato plants growing on the border of the experimental plot supplied with *Pongamia* seed cake were free from pest damage symptoms while plants located at a distance from the experimental plot were seriously affected by the pest. Since *Pongamia* cake has a characteristic odour like neem cake which was earlier shown to exert insect repellant properties due to its volatile constituents [21], we suspected that the strong odor of volatiles emanating from *Pongamia* cake could be responsible for its protective action. A thorough search of literature showed that no information exist on the nature of volatiles emitted from *Pongamia* seed and cake or their action on pests. Hence, this study was undertaken to determine the composition of headspace volatiles using Gas chromatography-mass spectrometry (GC-MS). The fours of the present work was to separate and identify the entire range of volatile compounds emitted by *Pongamia* seed and cake using GC and GC-MS for exploring the possible commercial application and use of *Pongamia* volatiles for insect pest control in horticultural crops through eco-friendly approach.

MATERIALS AND METHODS

Plant material

Mature pods of *Pongamia pinnata* (L) Pierre were collected from locally grown trees seeds were separated by breaking the pods and allowed to shade-dry for a month, after which they were crushed into bits. The kernel obtained after decorticating the seeds was crushed in a hydraulic press at a high pressure in a single step to extract the oil. The resulting cake was ground into a fine powder and used for the experiment. *Pongamia* seed powder was prepared by finely pulverizing the seeds in a tissue grinder immediately before use.

Isolation of volatile components

Extraction of headspace volatiles using solid phase micro extraction (SPME) was employed in the present study as previous studies had shown that such sampling could avoid interferences from nonvolatile matrix components [7,24]. Extraction of headspace volatiles of *Pongamia* seed and cake was performed as described earlier [23]. The SPME fiber coated with carboxan/ polydimethylsiloxane/ divinylbenzene (50/30 µm, CAR/PDMS/DVB) (Supelco, Bellefonte, PA, USA) was used for analysis owing to its high sensitivity for aroma compounds and excellent reproducibility. For sampling, 50 g each of seed and cake were homogenized with 100 ml double distilled water using a commercial blender. The slurry was transferred to a 250 ml conical flask to which 5 g of NaCl was added. Subsequently, the flask was sealed with a silicone rubber septum and kept at 37±1°C with continuous stirring for 20 min to allow for equilibration between the solution and the headspace. The fibre was exposed to the headspace of the sealed flask for 60 min. Prior to sampling, the fibre was preconditioned for 1hr at 260°C in the GC injection port as per the manufacturer's instructions.

Gas chromatography

GC-FID analysis was carried out using a Varian-3800 Gas chromatograph system equipped with manual SPME holder and a liner adapted to injector, on a VF-5 column (Varian, USA), 30 m × 0.25 mm id and 0.25ìm film thickness. The carrier gas was helium used at a flow rate of 1ml/min; injector temperature, 250°C and detector temperature, 270°C. The temperature program for column oven was as follows: The initial oven temperature was 50°C for 2 min, increased by 3°C /min up to 200°C, held for 3 min, increased further at 10°C /min up to 220°C and maintained constant for 8 min. For desorption, the SPME device was introduced in the injector port for chromatographic analysis and remained in the inlet for 12 min. Initially injection mode was splitless followed by split mode (1:20) after 1.5 minutes.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was performed on Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS-MS ion trap mass selective detector. Volatile compounds were separated on VF-5MS (Varian, USA) column (30 m × 0.25 mm id with 0.25 ìm film thickness) by applying the same temperature program as described above for GC-FID analysis. Mass detector conditions were: EI-mode at 70 eV with full scan range, 50–450 amu. The carrier gas was helium at a flow rate of 1ml/min; injector temperature, 250°C; ion source-temperature, 230°C; trap temperature, 220 °C and transfer line temperature, 250°C.

Identification of components

Volatile compounds were identified by comparing the mass spectra with the available libraries (Wiley and NIST-2007) and by retention indices (RI) computed in accordance with modified Kovats method [6,11] using a homologous series of n-alkanes (C_5 to C_{32}). Relative proportion of compounds in the mixture was calculated from the FID chromatogram and quantified as percent area. The total volatile production was estimated as the sum of the area of all GC-FID peaks in the chromatogram. All data are means of three independent determinations expressed as percentage of total GC-FID peak area.

RESULTS AND DISCUSSION

Results presented in Table 1 showed that the a total of 84 and 81 volatile compounds could be separated and identified from the headspace samples of *Pongamia* seed and cake, respectively. These compounds belonged to ten major groups namely, hydrocarbons (aliphatic and aromatic), terpenoids, alcohols, phenols, aldehydes and ketones, acids, esters, oxo compounds, sulphur and nitrogen containing compounds. The most abundant compound in both seed and cake was 2-chloroacetophenone (19.58%, 19.47% respectively).

There were significant differences in the proportion of terpenoids, sulphur compounds, phenols and nitrogenous compounds between the volatile components of seed and cake while others showed very little differences. The levels of sulphur compounds increased from 5.59% in seed to 17.18% in cake while phenols and terpenoids decreased from 22.33% in seed to 5.30% in cake and from 19.48% in seed to 12.84% in cake, respectively. The levels of nitrogenous compounds in cake (5.28%) was more than double compared to seed (2.31%). These changes in the composition of cake volatiles as against the seed could, perhaps be attributed to the action of seed enzymes on substrates which are released due to cellular damage during the extraction of oil from seed.

Terpenoids formed the largest group comprising 20 and 18 compounds in seed and cake, respectively followed by aldehydes and ketones having 17 and 16 compounds, and alcohols group consisting of 14 compounds in seed and 15 in cake. The proportion of hydrocarbon compounds in seed was 3.11% as against 3.32% in cake. Acids, esters, nitrogenous and oxo compounds together constituted 8.66% and 13.26% in seed and cake respectively. These results suggested that *Pongamia* seed and cake are rich sources of volatiles containing a number of compounds. Many of them including, limonene, α-copaene, α-gurjunene, aromadendrene and δ-cadinene are known to possess insecticidal activities. Limonene, particularly the (*R*)-(+)-enantiomer is most active as an insecticide [5]. The rare (+)-α-copaene found in small amounts in some plants is of economic significance because it is strongly attracting to an agricultural pest, the Mediterranean fruit fly, *Ceratitis capitata* [16]. The essential oil obtained from leaves and stem barks of the Southern Brazilian native *Drimys brasiliensis* Miers, a tree with medicinal properties, is reported to contain α-gurjunene (6.0%). Tests have shown that the oil containing α-gurjunene was lethal, killing 95-98% of the larvae of cattle tick, *Rhipicephalus* (Boophilus) *microplus* and the brown dog tick, *Rhipicephalus sanguineus* at the level of 3.125 µl/ml [18]. The essential oil of the fruits of *Eucalyptus globulus* contain aromadendrene is reported to

Table 1. Composition of *Pongamia* seed and cake volatiles

Compound identified	**RI calculated**	**RI reported**	**Seed (% Area)**	**Cake (%Area)**	**Mode of identification**
HYDROCARBONS (*Aliphatic and Aromatic*)					
Toluene	746	748	0.68	1.53	KI,MS
Styrene	890	895	0.18	0.21	KI,MS
3-Propyl-1-cyclohexene	944	NA	0.12	ND	MS
1,2,3-Trimethylbenzene	998	992	ND	0.13	KI,MS
p-Cymene	1032	1026	0.08	0.19	KI,MS
Azulene	1319	1311	0.27	0.41	KI,MS
Tetradecane	1396	1400	0.80	0.23	KI,MS
(-)-Isoledene	1438	NA	0.20	0.43	MS
4-Methylpentadecane	1558	NA	0.17	0.06	MS
Hexadecane	1604	1600	0.31	ND	KI,MS
2,6-Diisopropylnaphthalene	1721	1728	0.14	0.13	KI,MS
2,2',5,5'-Tetramethyl-1,1'-biphenyl	1793	NA	0.16	ND	MS
TERPENOIDS *(mono- and sesquiterpenoids)*					
α-Pinene	936	936	0.30	ND	KI,MS
Limonene	1031	1033	0.63	0.09	KI,MS
cis-Ocimene	1045	1046	0.13	ND	KI,MS
α-Cubebene	1338	1345	0.41	0.93	KI,MS
α-Copaene	1354	1375	1.76	0.81	KI,MS
Longifolene	1402	1413	0.18	0.10	KI,MS
α-Gurjunene	1416	1403	0.39	0.15	KI,MS
β-Caryophyllene	1419	1427	1.81	1.41	KI,MS
(+)-Aromadendrene	1425	1440	0.34	0.41	KI,MS
γ-Muurolene	1468	1473	0.49	0.41	KI,MS
Allo-Aromadendrene	1461	1466	0.11	0.13	KI,MS
β-Bisabolene	1501	1485	0.16	0.19	KI,MS
β-Selinene	1488	1492	0.67	1.07	KI,MS
Valencene	1490	1497	3.28	1.80	KI,MS
γ-Selinene	1496	1532	0.14	0.13	KI,MS
α-Muurolene	1498	1498	2.14	1.49	KI,MS
γ-Cadinene	1512	1510	3.20	1.98	KI,MS
δ-Cadinene	1518	1524	2.37	1.28	KI,MS
(-)-Calamenene	1522	1524	0.76	0.26	KI,MS
α-Calacorene	1542	1537	0.21	0.20	KI,MS
ALCOHOLS					
Hept-6-en-3-yn-1-ol	841	NA	0.17	ND	MS
3-*cis*-5-Heptadien-1-ol	924	921	ND	0.06	KI,MS
4-Terpineol	1179	1191	0.13	0.38	KI,MS
(Z)-3-Nonen-1-ol	1119	1126	0.47	2.02	KI,MS
α-Methylbenzeneethanol	1215	1210	ND	0.40	KI,MS
3-Decyn-1-ol	1268	NA	0.09	0.30	MS

3,4-Dimethoxyphenylmethyl alcohol	1411	NA	0.16	0.40	MS
4,8-Bis(hydroxymethyl)tricyclo[5.2.1.0(2,6)]decane	1478	NA	0.11	0.09	MS
Methylisoeugenol	1484	1491	0.10	0.57	KI,MS
1-Tetradecanol	1659	1654	0.73	0.15	KI,MS
2,6,8-Trimethylpyrido[3,4-d]pyrimidin-4-ol	1746	NA	0.55	0.42	MS
Viridiflorol	1584	1585	0.24	0.21	KI,MS
Longiborneol	1593	1592	0.25	0.05	KI,MS
Cubenol	1627	1641	0.65	0.66	KI,MS
α-Cadinol	1635	1638	4.77	3.74	KI,MS
(-)-δ-Cadinol	1639	1647	0.42	0.27	KI,MS
ALDEHYDES AND KETONES					
Nona-3,5-dien-2-one	1072	NA	0.53	ND	MS
cis-6-Nonenal	1141	1161	0.18	0.31	KI,MS
trans-6-Nonenal	1152	1150	0.09	0.11	KI,MS
2-Hydroxy-3-methyl benzaldehyde	1172	NA	0.25	1.56	MS
p-Isopropylbenzaldehyde	1236	1239	0.41	0.92	KI,MS
p-Anisaldehyde	1246	1247	0.04	0.20	KI,MS
Hydroxycitronellal	1270	1273	ND	0.13	KI,MS
2-Chloroacetophenone	1285	1283	19.58	19.47	KI,MS
4-Methoxysalicylaldehyde	1375	1396	0.13	0.12	KI,MS
β-Damascenone	1376	1391	0.19	0.73	KI,MS
2-Carboxybenzaldehyde	1421	NA	0.15	ND	MS
3,4-dimethoxy-Benzaldehyde	1428	NA	0.34	0.75	MS
3,4,7-Trimethyl-1-indanone	1519	NA	0.10	0.67	MS
2,5-Dimethylterephthalaldehyde	1535	NA	1.32	1.54	MS
(-)-Vermelone	1561	NA	0.68	0.70	MS
Benzophenone	1602	1612	1.16	0.70	KI,MS
2-(hexylthio) decanal	1852	NA	0.06	0.86	MS
(9Z)-9,17-Octadecadienal	1958	NA	3.74	3.15	MS
ACIDS					
Benzoic acid	1165	1180	0.20	0.24	KI,MS
n-Hexadecanoic acid	1941	1957	2.87	4.14	KI,MS
Octadecanoic acid	2061	2075	0.58	0.44	KI,MS
ESTERS					
Ethyl benzoate	1159	1170	0.28	0.26	KI,MS
Isopropyl benzoate	1192	1195	0.25	0.53	KI,MS
Ethyl (3E)-3-nonenoate	1272	NA	0.39	0.19	MS
Methyl 3-methoxy-4-methylbenzoate	1342	NA	0.37	0.54	MS
Phenyl benzoate	1598	NA	0.65	0.26	MS
Methyl 6-octadecenoate	2076	2081	0.40	1.05	KI,MS
OXO-COMPOUNDS					
o-Dimethoxybenzene	1156	1149	ND	0.13	KI,MS
3,5-Dimethoxytoluene	1257	1264	0.08	0.13	KI,MS
1-Acetyl-4,6,8-trimethylazulene	1642	NA	0.28	0.07	MS

SULPHUR COMPOUNDS					
2,5-Dimethylthiophene	865	868	0.16	0.05	KI,MS
Benzoyl thiol	1212	NA	1.44	9.88	MS
Benzyl Isothiocyanate	1354	1361	0.77	2.55	KI,MS
2,7-diethyl-Benzothiophene	1523	NA	2.90	4.56	MS
1-Ethyldibenzothiophene	1738	NA	0.32	0.14	MS
NITROGEN COMPOUNDS					
o-Tolyl isocyanide	1041	NA	0.08	0.25	MS
Nitrocyclohexane	1068	NA	0.11	0.08	MS
3-Nitrobenzaldehyde	1322	1315	2.12	4.95	KI,MS
PHENOLS					
Guaiacol	1074	1092	17.00	5.00	KI,MS
p-Vinylguaiacol	1302	1312	5.00	0.21	KI,MS
5-Methyl-2-propylphenol	1311	NA	0.33	0.09	MS
UNIDENTIFIED			3.07	6.50	

exert marked inhibition against multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) *Enterococcus faecalis*. [15]. The hydrodistilled essential oils of the leaves and twigs of *Litsea mushaensis* and *L. linii* containing β-selinene (15.7%) and α-selinene (15.5%), were shown to have excellent antimicrobial and anti-wood-decay fungal activity, superior to the other oils [4] . Essential oils from fresh leaves of *Vitex negundo* were found to contain δ-guaiene, carryophyllene epoxide and ethyl-hexadecenoate and flowers contained α-selinene, germacren-4-ol, carryophyllene epoxide and (E)-nerolidol which were active against *B. subtilis* and *E. coli* [9]. Similarly, fruit essential oil of *Illicium simonsii* (Aquifoliaceae) that contained â-caryophyllene (10.30%), δ-cadinene (9.52%), and ME (8.94%) as major components had strong fumigant and contact toxicities against adults of the maize weevil, *Sitophilus zeamais*, with LC_{50} values of 14.95 mg/L air and 112.74 µg/adult, respectively [3]. Thus, from the literature information reviewed here, it is evident that *Pongamia* volatiles contain several bio-active compounds contributing to its insecticidal activity. This finding being reported for the first time in this paper confirms our observations on the pesticide activity of *Pongamia* volatiles and provides a strong basis for application of *Pongamia* volatiles for crop protection. The volatiles emitted by *Pongamia* cake also appear to be similar to neem cake volatiles containing a large number of biologically active volatile compounds that are effective against a wide spectrum of plant pests and pathogens [1, 12] Previous studies in our lab had shown that volatiles of neem cake were effective in controlling diamondback moth, *Plutella xylostella* (L) of cabbage [12]. Taking into account the occurrence of a large number of compounds in *Pongamia* volatiles endowed with insecticidal activity, it may be possible to identify many more biologically active volatile components of *Pongamia* for application in protection of crops against pests and diseases. Needless to emphasize, the plant-derived products have the advantage of being not only effective against pests but are also biodegradable, harmless to non-target and beneficial organisms, non-polluting and non-toxic unlike chemical pesticides.

To sum up, the data presented in this paper on the volatile composition of seed and cake of *Pongamia* by GC-MS analysis, for the first time, could be utilized to evolve an eco-safe formulation, replacing chemical pesticides, for the effective and efficient management of pests and pathogens of crops.

ACKNOWLEDGEMENTS

The authors wish to thank the Director of the institute for providing facilities for the study and Mr. S.S.A. Qazi for the skilled assistance in the analysis of samples.

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