J Wood Sci (2005) 51:185–188
DOI 10.1007/s10086-004-0640-4

NOTE

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Components of the essential oils of *Azadirachta indica* A. Juss, *Azadirachta siamensis* Velton, and *Azadirachta excelsa* (Jack) Jacobs and their comparison

Received: November 17, 2003 / Accepted: March 26, 2004

Abstract The components of the essential oils from seeds of *Azadirachta indica*, *Azadirachta siamensis*, and *Azadirachta excelsa* were studied by gas chromatography and gas chromatography-mass spectrometry. The main components of *A. indica* oil were hexadecanoic acid (34.0%), oleic acid (15.7%), 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine (11.7%), methyl oleate (3.8%), and eudesm-7(11)-en-4-ol (2.7%). The major components of *A. siamensis* oil were hexadecanoic acid (52.2%), tricosane (10.5%), tetradecanoic acid (6.8%), oleic acid (4.9%), and pentacosane (4.9%). *Azadirachta excelsa* oil contained oleic acid (31.3%), hexadecanoic acid (14.2%), octadecanoic acid (13.0%), 4-octylphenol (9.7%), and *O*-methyloximedecanal (6.8%) as the main constituents. The essential oils from *A. indica*, *A. siamensis*, and *A. excelsa* were found to contain fatty acids (52.6%–72.3%) as major components. The minor components of the oils were *n*-alkanes, aromatics, esters, sulfur and nitrogen compounds, and terpenoids. Differences in oil composition were observed between the three species.

Key words *Azadirachta indica* · *Azadirachta excelsa* · *Azadirachta siamensis* · Neem · Essential oil

Introduction

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is a tropical and subtropical species indigenous to India and Southeast Asia. Various neem tree parts have been used by natives in cooking, in folk medicine, and as natural pesticides.[1] Ripening neem fruits and expressed neem seed oil give off a strong alliaceous (garlic-like) odor, and some of the reputed medicinal efficacies of neem oil have been attributed to the sulfurous compounds that it contains.[1–4]

The sadao chang tree (*Azadirachta excelsa* (Jack) Jacobs), known as tiem in Thailand and sentang in Malaysia, is fast growing and economically valuable not only for timber but also for its medicinal and pesticidal properties. Young leaves and fresh flowers can be used as food, and the seeds contain an important chemical, known as azadirachtin, which is used as a natural insecticide. It occurs in Thailand and probably Myanmar (formerly Burma) and its common names are Thai neem and dibble neem, because young leaves and flowers are consumed as a vegetable.

The volatile oil component of *A. indica* has been reported.[4–6] However, no analysis of the essential oils from *Azadirachta siamensis* and *Azadirachta excelsa* has been reported. The present investigations were designed to identify the essential oil components of *Azadiractha* species and to compare the composition of each essential oil.

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Materials and methods

Plant materials

Plant materials were collected in India (*Azadirachta indica*), Thailand (*Azadirachta siamensis*), and the Philippines (*Azadirachta excelsa*) in July 2002. Voucher specimens were deposited in the Department of Global Agricultural Science, Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Essential oils of neem

Broken neem seeds were subjected to hydro distillation (a kind of steam distillation) for 3 h using a modified essential oil collector standardized by the American Oil Chemists' Society. The yields of oils from seeds of *A. indica*, *A. siamensis*, and *A. excelsa* were 0.028%, 0.018%, and 0.028%, respectively.

Gas chromatography

Gas chromatography (GC) was carried out using a Shimadzu GC-17B instrument equipped with a flame ionization detector (FID) on a capillary column (TC-FFAP fused silica, film thickness 0.4 μm, 60 m $\times$ 0.25 mm) prepared by GL Science. The column temperature was programmed over the range of 60°–220°C at the rate of 3°C/min and held at 220°C for 30 min. The injector and detector temperatures were both 220°C. Helium was used as a carrier gas and the flow rate was 1.0 ml/min. Quantitative data were obtained from FID area percentages without the use of correction factors.

Gas chromatography-mass spectrometry (GC-MS)

The MS was carried out with a Shimadzu GCMS-QP5050 mass selective detector. GC conditions were the same as in the above-mentioned method. The detector interface temperature was set at 220°C, to which the actual temperature in the MS source nearly reached, and the ionization voltage was 70 eV. Helium was used as a carrier gas and the flow rate was 1.0 ml/min.

Identification of components

The components of the oils were identified by comparing their mass spectra with those of a computer library (NIST 12 and 62), with authentic compounds, or with data published in the literature.[7] They were confirmed by comparing their retention indices (RI) with those of authentic compounds or published data.[8]

Results and discussion

The yields of essential oils collected by hydro distillation from the three neems of *Azadirachta indica, Azadirachta siamensis*, and *Azadirachta excelsa* were 0.018%–0.028% (w/w). The constituents of the oils of the neems are given in Table 1, together with experimental RI in polar columns, including their percentage of peak area. Distinct qualitative and quantitative differences were observed in the neem oils studied.

The main components of *A. indica* oil were hexadecanoic acid (34.0%), oleic acid (15.7%), 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine (11.7%), methyl oleate (3.8%), and eudesm-7(11)-en-4-ol (2.7%). The major constituents of *A. siamensis* oil were hexadecanoic acid (52.2%), tricosane (10.5%), tetradecanoic acid (6.8%), oleic acid (4.9%), and pentacosane (4.9%). Those of *A. excelsa* oil were oleic acid (31.3%), hexadecanoic acid (14.2%), octadecanoic acid (13.0%), 4-octylphenol (9.7%), and *O*-methyloximedecanal (6.8%).

The main constituent of the neem oils was fatty acids, the total contents of which in the oils of *A. indica, A. siamensis*, and *A. excelsa* were 52.6%, 72.3%, and 67.4%, respectively.

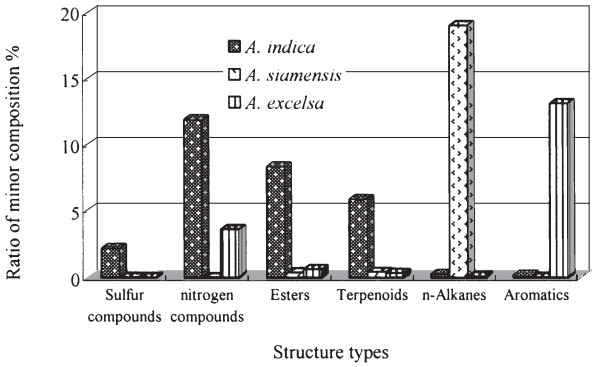

Fig. 1. Minor composition of *Azadirachta indica*, *Azadirachta siamensis*, and *Azadirachta excelsa* oils

Azadirachta indica oil was characterized by a high content of hexadecanoic acid (34.0%) and low content of oleic acid (15.7%), while the *A. excelsa* oil possessed a lower content of hexdecanoic acid (14.2%) and higher content of oleic acid (31.3%). *Azadirachta siamensis* had hexadecanoic acid as the main compound, which accounted for about 50% of the oil.

The neem oils were characterized by the minor components of the six structure types *n*-alkanes, aromatics, esters, sulfur and nitrogen compounds, and terpenoids, as shown in Fig. 1. In *A. indica* and *A. excelsa* seed oils, the five nitrogen compounds: 2,6-diethylpyridine [MS *m/z* 135 (M+, 61), 134 (100), 120 (3), 107 (17), 93 (5), 77 (11), 65 (3), 44 (18)]; 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine [*m/z* 205 (M+, 29), 172 (1), 140 (3), 131 (12), 98 (43), 84 (15), 74 (32), 68 (26), 58 (100), 45 (32), 41 (84)]; 1H-pyrazole [*m/z* 68 (M+, 100), 41 (54)]; 1H-benzotriazole [*m/z* 119 (M+, 100), 98 (3), 91 (94), 64 (40), 63 (31), 52 (12), 41 (33)]; and dodecanamide [*m/z* 199 (M+, 1), 156 (3), 147 (3), 128 (4), 114 (3), 100 (3), 86 (5), 72 (25), 59 (100), 43 (25)], were also found. *Azadirachta indica* oil contained esters, sulfur compounds, and terpenoid, in contrast to the essential oils from *A. excelsa* and *A. siamensis*, which contained very small amounts of those. It is interesting to note that aromatic compounds were identified in the *A. excelsa* oil. *Azadirachta siamensis* oil contained *n*-alkanes as the main class of constituents.

Conclusions

The major components of the oils of *Azadirachta* species were fatty acids. The essential oils of the seed of *Azadirachta* species were predominantly composed of fatty acids. The fatty acid present in the largest amounts for *Azadirachta indica* and *Azadirachta siamensis* was hexadecanoic acid. In case of *Azadirachta excelsa*, oleic acid was the most abundant fatty acid.

Sulfur and nitrogen compounds, terpenoids, aromatic compounds, aliphatic esters, and *n*-alkanes were also present as minor components. Each oil of the *Azadirachta* species contained its characteristic compounds, namely, *A.*

Table 1. Compounds of the essential oils from three *Azadirachta* neems

Compounds	RI[a]	*Azadirachte indica*	*Azadirachte siamensis*	*Azadirachte excelsa*
Sabinene[b]	1133	0.08		
2-Methyl-2-pentanal	1177	0.23		
Limonene[b]	1210	0.37		
4-Hydroxy-4-methyl-2-pentanone	1375	0.25		0.20
1-Octen-3-ol	1460	0.13		
Pentadecane[b]	1500	0.13		
2,6-Diethylpyridine	1533	0.20		
1-Octanol	1567	0.14		
Bergamotene	1588	0.25		
Hexadecane[b]	1600	0.13		
Valencene	1719	0.29		
Aristolene	1727	0.22		
β-Bisabolene	1731	0.32		
cis-3,5-Diethyl-1,2,4-trithiolane	1756	0.42		
ar-Curcumene	1780	0.18		
trans-3,5-Diethyl-1,2,4-trithiolane	1797	0.72		
2-Tridecanone	1818	0.16		
Neryl acetone	1865	0.20		
5,6-Dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine	1977	11.68		
Unknown[c]	1984	1.36		
Phenol[b]	2015		1.58	
2-Pentadecanone	2029	0.16		
Heneicosane[b]	2100	0.33		
Unknown[d]	2004	0.60		
Italicene	2141	0.69		
Unknown[e]	2193	0.70		
Docosane[b]	2200	0.16	0.08	
Thymol[b]	2200	0.31		
Cadalene	2224		0.33	
Methyl palmitate	2226	1.34		
Globulol	2238	0.41		
1H-Pyrazole	2239		2.10	
Eudesm-7(11)-en-4-ol	2239	2.68		
Ethyl palmitate	2263	0.23	0.38	
Syringol[b]	2284		0.12	
Tricosane[b]	2300	10.48		
3,5-Di-*t*-butylphenol	2328	0.16		
Propyl palmitate	2348	0.07		
1,3,5-Trithiolane	2360	1.05		
Tetracosane[b]	2400	0.74		
Coumaran	2415		0.42	
8-Methylhydrocoumarin	2425		0.16	
Methyl octadecanoate	2433	0.23		0.08
Nonanoic acid	2442	0.25		
Benzoic acid	2449		1.23	
Methyl oleate	2455	3.80		0.54
Ethyl palmitate	2589	0.41		
Dodecanoic acid	2495	1.36	6.13	
Abietatriene[b]	2498	0.29		
Pentacosane[b]	2500	4.88		
Methyl linolenoate	2503	1.12		
1-Octadecanol	2509	0.78		
Hexacosane[b]	2600	0.21		
O-Methyloximedisdecanol	2611		6.82	
4-Octylphenol	2620		9.73	
1H-Benzotriazole	2629		0.79	
Tridecanoic acid	2651	1.39		
Methyl linolenoate	2660	1.02		
Heptacosane[b]	2700	1.64		
Tetradecanoic acid	2708	6.75	0.15	
Anthracene	2746	0.42		
Methyldibenzothiophene	2775	0.16		
Dodecanamide	2784		0.72	
Pentadecanoic acid	2809	1.23		
Nonacosane[b]	2900	0.54		
Hexadecanoic acid	2920	34.01	52.18	14.20
9-Hexadecenoic acid	2950	1.78		
Octadecanoic acid	3063	2.93		13.00
Oleic acid	3079	15.67	4.93	31.29
Linoleic acid[b]	3107	2.40	2.59	

[a] Retention indices (RI) on TC-FFAP
[b] Components identified with authentic compounds
[c] MS *m/z*: 204[M]+ (22), 187 (15), 175 (23), 161 (28), 147 (32), 133 (30), 121 (30), 106 (95), 93 (100), 79 (93), 60 (58), 43 (82), 41 (95)
[d] MS *m/z*: 220[M]+ (10), 208 (5), 189 (12), 177 (20), 161 (23), 151 (100), 133 (52), 123 (45), 107 (82), 91 (71), 79 (65), 55 (76), 41 (97)
[e] MS *m/z*: 204[M]+ (35), 193 (81), 180 (19), 161 (100), 152 (42), 138 (22), 121 (76), 98 (47), 95 (78), 81 (38), 58 (32), 43 (93)

indica contained sulfur compounds, esters, and terpenoids larger in higher levels than other species, while *A. siamensis* contained *n*-alkanes and *A. excelsa* contained aromatics at higher levels than in the other species.

In these seed oils, five nitrogen compounds were also found. *Azadirachta indica* contained a large amount of 5,6-dihydro-2,4,6-triethyl-(4H)-1,3,5-dithiazine, and the other three compounds were present in small amounts in *A. excelsa*. *Azadirachta siamensis* contained no nitrogen compounds.

Distinct qualitative and quantitative differences were observed in the oils of three neems of *Azadirachta* species studied, which suggests that the components of essential oils from neems can be applied to judge product quality.

Acknowledgment We thank Aprot Co. Ltd. for plant material supply.

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