SHORT COMMUNICATION

Essential oil of the flowers of *Azadirachta indica* (Meliaceae)

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Abstract

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Essential oil of the flowers of *Azadirachta indica* was obtained at 0.001% yield by steam distillation. It mainly consisted of α-cubebene (3.04%), copaene (7.03 %), humulene (3.7 %), δ-cadinene (9.43 %) and a number of sesquiterpenes. The oil also contained a series of unidentified organosulphur compounds. These organosulphur compounds were picked up by the S^{34} isotope. The oil exhibited antimicrobial activity against *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) and *Microsporum gypseum* (Clinical isolated).

Key words : *Azadirachta indica*, flowers, steam distillation, volatile constituents, organosulphur

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บทคัดย่อ

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น้ำมันหอมระเหยในดอกสะเดา (*Azadirachta indica*)
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น้ำมันหอมระเหยจากดอกสะเดาที่ได้จากการกลั่นด้วยไอน้ำ (0.001%) องค์ประกอบส่วนใหญ่ ได้แก่ α-cubebene (3.04%), copaene (7.03%), humulene (3.7%), δ-cadinene (9.43%) และ sesquiterpenes อีกจำนวนหนึ่ง นอกจากนี้ ในน้ำมันยังประกอบด้วยสารที่มีกำมะถันเป็นองค์ประกอบซึ่งได้พิสูจน์เอกลักษณ์โดยอาศัยพีคไอโซโทปของกำมะถัน (S^{34}) น้ำมันนี้สามารถต้านเชื้อ *Bacillus subtilis* (ATCC 6633) *Candida albicans* (ATCC 10231) และ *Microsporum gypseum* (แยกจากผู้ป่วย)

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Azadirachta indica A. Juss. (neem), *Meliaceae*, is widely grown in South Asia, South-east Asia and West Africa. It is an evergreen tree, 12-15 m in height, with pinnate leaves up to 3-10 cm long. Panicles of small, yellow-white, mildly pungent flowers appear in winter (November to December in Thailand). Fruits are berry-like with yellow to red colour. In Thailand, the flowering tops are consumed fresh with chilli dipping as a vegetable and used as a bitter tonic (Jain and De Fillipps, 1991; Bruneton, 1995; Brown, 1995). Other parts of the plant, root, leaves, and bark, have been used for medicinal purposes. Seeds have been used as an antianthelmintic and insecticide. The volatile components from the flowers of this plant have not been investigated.

Materials and Methods

Plant material

Flowers of *A. indica* were collected in early November 2002, in Khon Kaen, Thailand. Young flower buds, flowers and pedicels were steam distilled overnight using the volatile oil distillation apparatus as described in British Pharmacopoeia, 1988. The volatile oil was collected in xylene. The xylene portion changed the colour from colourless to yellow. The yield was 0.001% w/w calculated based on 3 kilograms of the fresh flowers. The xylene solution of the steam distillate was diluted 25 µL to 1 ml with chloroform and injected into a GC/MS.

Gas chromatography system

A Fisons 8000 series gas chromatography equipped with a mass spectrometer detector, MD 800 and a DB-Wax fused silica column (30 m x 2.5 mm, 0.25 µm film thick) was used. The temperature was programmed from 40ºC (after 5 min) to 250ºC at 3ºC/min and stayed for 10 minutes. Helium was used as a carrier gas at the pressure of 50 Kpa and the MS was operated at 70 eV with ion source temperature of 250ºC

Identification of the components

Component identification was carried out by a reversed fitting with the NIST standard spectra and from linear retention index values (Davies, 1990). Sulphur compounds were picked up by the S^{34} isotope peak of the parent ion. The compounds were listed as MWS (i.e. 112S is a compound of MW 112 which contains sulphur).

Antimicrobial activities

Inocula of bacterial and fungal cultures were prepared according to the standard method (Acar and Goldstein, 1991). An agar diffusion method was used to determine the antibacterial and anti-fungal activities. The oil solution was diluted with sterilized 0.5% Tween 80 and the solvent used in

collecting the oil was employed as a control. The test cultures were *Staphyllococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC29212, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and *Microsporum gypseum* (Clinical isolated).

Results and Discussion

The volatile components of the flowers of *A. Indica* had a strong sulphury smell. The main components were sesquiterpenes, δ-cadinene (9.43%), copaene (7.03%), humulene (3.70%), δ-cubebene (3.04%), and some minute amount of others as listed in Table 1. Ethyl laurate and ethyl ester of high molecular fatty acids and high molecular weight of hydrocarbons were also found. There were 21 organosulphurs detected, in which a number of unidentified components were picked up by the S^{34} isotope peak of the parent ions. The organosulphur components found were similar to those found in the neem seed oil obtained by steam distillation (Mubarak and Kulatilleke, 1992; Balandrin *et al*., 1988). All components were identified by the MS data and the linear retention index.

The peak at the retention time of 19.52 minutes (m/z 166) is suggested to be $HSSSC_5H_9$ according to the following MS data: m/z (rel. int): 41 ($C_3H_5^+$, 83), 45 ($CH{\equiv}S^+$, 32), 47 ($S^{\bullet+}CH_3$, 11), 59 ($SC_2H_3^+$, 71), 60 ($S^{\bullet+}C_2H_4$, 86), 64 ($2S^{\bullet+}$, 59), 69 ($C_5H_9^+$, 94), 73 ($C_3H_5S^+$, 50), 74 ($C_3H_6S^{\bullet+}$, 40), 92 ($C_2H_4S_2^{\bullet+}$, 48), 101 (M^+-SSH, 53), 102 (M^+-2S, 69), 106 (M-C_2H_4S, 34), 166 (M^+, 100). The isotope peaks at m/z 168 (13.2% of m/z 166) and 167(8.1% of m/z 166) indicated the existence of 3 sulphur atoms and 5 carbons and the compound could be

Table 1. Composition of the volatiles oil from the flowers of *Azadirachta indica*, listed in elution order of the DB-wax column.

Components	R_t	LRI	%	Method of identification
3,4-Dimethylthiophene	8.85	1250	0.8	LRI, MS
Organosulphur (122S)	10.75	1322	0.8	MS
Dipropyl disulphide	12.2	1378	0.29	LRI, MS
Nonanal	12.66	1395	0.21	MS
Propyl propenyl disulphide	13.66	1436	0.24	LRI, MS
Organosulphur (150S)	14.08	1453	0.76	MS
α-Cubebene	14.23	1459	3.04	LRI, MS
δ-Elemene	14.52	1471	0.61	LRI, MS
α-Copaene	15.08	1493	7.03	LRI, MS
Organosulphur (148S)	15.7	1520	0.02	MS
α-Gurjunene	15.59	1530	0.11	LRI, MS
Organosulphur (148S)	16.29	1530	0.02	MS
Linalool	16.5	1554	0.37	LRI, MS
(A sesquiterpene) $C_{15}H_{24}$	17.02	1576	0.26	MS
(A sesquiterpene) $C_{15}H_{24}$	17.37	1591	0.43	MS
Caryophyllene	17.51	1597	4.32	LRI, MS
Organosulphur (152S)	17.59	1600	0.02	MS
Aromadendrene	17.75	1608	0.62	LRI, MS
(A sesquiterpene) $C_{15}H_{24}$	18.12	1624	2.85	MS
(A sesquiterpene) $C_{15}H_{24}$	18.49	1641	0.45	MS

Rt = retention time
LRI = linear retention index

Table 1. (Continued)

Components	R_t	LRI	%	Method of identification
(A sesquiterpene) $C_{15}H_{24}$	18.59	1662	1.29	MS
Allo-aromadendrene	18.61	1646	1.18	LRI, MS
Humulene	19.15	1671	3.7	LRI, MS
Organosulphur (166S)	19.52	1687	1.57	MS
(A sesquiterpene) $C_{15}H_{24}$	19.59	1691	1.29	Ms
Viridiflorene	19.75	1698	0.7	LRI, MS
Organosulphur (166S)	19.99	1709	1.98	MS
(A sesquiterpene) $C_{15}H_{24}$	20.23	1720	1.51	MS
α-Muurolene	20.38	1727	1.14	LRI, MS
Bicyclogermacrene	20.56	1736	0.01	LRI, MS
δ-Cadinene	21.11	1762	9.43	LRI, MS
(A sesquiterpene) $C_{15}H_{24}$	21.22	1767	0.02	MS
cis-3,5-Diethyl-1,2-4-trithiolane (180S)	21.39	1775	8.82	LRI, MS
Cadina-1, 4-diene	21.62	1785	2.82	LRI, MS
trans-3,5-Diethyl-1,2,4-trithiolane (180S)	21.83	1795	8.85	LRI, MS
2-Tridecanone	22.22	1814	0.7	MS
Calamene	22.63	1834	1.66	LRI, MS
Ethyl ester of C_{12} acid	22.89	1847	0.45	MS
α-Calacorene	24.28	1916	0.55	LRI, MS
Palustrol	24.6	1933	0.16	LRI, MS
Ledol	26.55	2035	0.58	LRI, MS
An oxygenated sesquiterpene ($C_{15}H_{26}O$)	26.62	2039	0.01	MS
Nerolidol	26.75	2046	0.26	LRI, MS
Ethyl laurate	26.87	2053	0.82	MS
Cubenol	27.05	2063	0.79	LRI, MS
Epicubenol	27.22	2072	0.52	LRI, MS
Globulol	27.41	2083	0.65	LRI, MS
Viridiflorol	27.59	2093	0.60	LRI, MS
Organosulphur (250S)	28.25	2129	0.52	MS
Organosulphur (222S)	28.52	2144	0.97	MS
Organosulphur (236S)	28.84	2161	1.62	MS
τ-cadinol	29.12	2177	0.23	LRI, MS
Organosulphur (212S)	29.23	2183	0.24	MS
τ-muurolol + 212S	29.35	2189	0.25	MS
Organosulphur (212S)	29.58	2201	0.24	MS
Ethyl ester of C_{16} acid	29.67	2204	0.30	MS
Ethyl ester of C_{16} acid	30.5	2234	0.35	MS
Organosulphur (298S)	31.92	2285	0.20	MS
Organosulphur (S)	32.07	2291	0.18	MS
Organosulphur (254S)	33.15	-	0.35	MS
Acid (200)	34.31	-	0.35	MS
Hydrocarbon	34.38	-	0.5	MS
Phytol	36.45	-	0.30	MS
Hydrocarbon	38.09	-	0.25	MS

Rt = retention time
LRI = linear retention index

an artifact due to the vigorous extraction and the high temperature GC conditions. This compound had not been reported elsewhere.

The oil showed no antibacterial activity against *S. aureus, E. faecalis, E. coli* and *P. aeruginosa*. However, it exhibited a moderate the clear zone on *B. subtilis* (10.5±0.5 mm), *C. albicans* (14±0.5 mm) and *M. gypseum* (11.0±1.0 mm).

Conclusion

Volatile oil of the neem flowers contained a number of sulphur components which are responsible for the sulphury odour. The oil showed activities against some bacteria and fungi.

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