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Medicinal used plants from India: analysis of the essential oils of *Sphaeranthus indicus* flowers, roots and stems with leaves

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Abstract: The essential oils of flowers, roots and herb (stems with leaves) of *Sphaeranthus indicus* Kurz. (Asteraceae) from southern India were investigated by gas chromatographic-spectroscopic (GC-FID and GC-MS) and olfactoric methods to identify those compounds responsible for the characteristic odor as well as partly for the folk medicinal use of this plant. More than 95 volatiles were found to be constituents of the three essential *S. indicus* oils with following composition of main components: flower oil: β-eudesmol (21.4%), 2,5-dimethoxy-p-cymene (16.2%), β-caryophyllene (7.8%), τ-cadinol (7.2%), caryophyllene oxide (6.9%) and α-eudesmol (4.5%); root oil: 2,5-dimethoxy-p-cymene (28.3%), τ-cadinol (25.3%), (*Z*)-arteannuic alcohol (10.1%), β-maaliene (3.9%) and caryophyllene oxide (3.1%); herb oil: 2,5-dimethoxy-p-cymene (27.0%), τ-cadinol (12.5%), β-eudesmol (9.1%), α-eudesmol (7.0%) and caroyphyllene oxide (4.7%). In addition, the odor impressions of the samples are described and the possible use of the essential *Sphaeranthus indicus* oils in medicinal, cosmetic and food flavoring discussed.

Key Word Index: *Sphaeranthus indicus*, Asteraceae, essential oils, flowers, roots, herb, odor, Indian folk medicine

Introduction: *Sphaeranthus indicus* Kurz. (Asteraceae) is an important plant, used in Indian folk medicine especially for its antiseptic, antitussive and tonic effects[1-4]. While various solvent extracts were investigated for their biological active compounds (mainly sesquiterpenoids)[4-10], the essential oils of *S. indicus* plants and/or plant parts were studied very rarely[1,2,11,12].

In continuation of our scientific work on analysis of essential oils used in traditional Indian medicine, also this research project was started to identify the volatiles of essential oils of different plant parts (flowers, roots and herb) of *Sphaeranthus indicus* and to discuss the constituents responsible for the characteristic aroma of these oils, their possible biological effects as well as applications in perfumery and cosmetics. To the best of our knowledge, no data on the composition of essential *S. indicus* oils from southern India are available until now.

Experimental:

Plant Material: The plants of *Sphaeranthus indicus* were collected on the Calicut University campus (Paddy field) in May 2000 and the plant material was identified by Dr.A.K. Pradeep, Department of Botany, Calicut University of Kerala. A voucher specimen is deposited in the specially maintained Herbarium of the Department of Chemistry at Calicut University (voucher-no. 35). The flowers, roots and herb of *S. indicus* were separated from the plant manually and dried at room-temperature for 14 days. After this, the three plant parts were powdered using an electric grinder each. The weights of powdered products were as follows: flowers: 650 g, roots: 250 g, herb: 1000 g.

Essential Oil Extractions: The powdered *S. indicus* flowers, roots and herb were taken into a 10 L Erlenmeyer-flask and then 5 L of water was added, before steam-distillation for 3.5 hours each. The essential oils were extracted with diethyl ether as solvent and dried over anhydrous sodium phosphate. Yields were found to be 0.31 g (0.05%) of an orange coloured essential flower oil, 0.30 g (0.12%) of a pale-yellow root oil and 0.44 (0.04%) g of a red-orange herb oil.

Olfactoric evaluations: The essential oils were diluted with dichloromethane, 10µl placed on a commercial odor-strip (Dragoco Co.) and the odor characterized by professional perfumers.

Gas Chromatography:- GC analyses were carried out using a Shimadzu GC-14A with FID and the integrator C-R6A-Chromatopac and a Varian GC-3700 with FID and the integrator C-R1B-Chromatopac (Shimadzu Co.). As columns a 30mx0.32mm bonded unpolar FSOT-RSL-200 fused silica (film-thickness: 0.25µm, Biorad Co.) and a 30mx0.32mm bonded polar Stabilwax (film-thickness: 0.50µm; Restek Co.) were used. Additional parameters as follows:

Carrier gas: hydrogen; injector-temperature: 250°C; detector-temperature: 320°C; temp.-programs: 40°C/ 5min. to 280°C/ 5min. with a heating-rate of 6°C/ min.; quantification by %-peak-area-calculations (unpolar column);
Some single components could be identified by co-injection of pure compounds and correlation of their retention-times (using Kovats indices) with published data[13-16].

Gas chromatography - Mass spectrometry:- The samples were analyzed by the GC-MS systems Shimadzu GC-17A with QP5000 and the data system Compaq-ProLinea (class5k-software), Hewlett-Packard GC-HP5890 with HP-5970MSD and PC-Pentium (Böhm Co., ChemStation-software) and Finnigan MAT GCQ with data system Gateway-2000-PS75 (Siemens Co., GCQ-software). Additional parameters as follows: Carrier gas: helium; injector-temperature: 250°C; interface-heating: 300°C; ion-source-heating: 200°C; EI-mode; scan-range: 41-450amu; other parameters see GC-FID-part.
For compound identifications Wiley-, NBS- and NIST-library spectra (on-line) as well as reference MS-spectra data[15,17-25] were used.

Results and discussion: The essential *Sphaeranthus indicus* oils from southern India were olfactorically evaluated as follows: warm-spicy, dry-woody-medicinal, borneol-note, floral (direction of rose) and fruity side-note (flower oil); warm-herbal, dried fruits-like top-notes (direction of plums and apricot, cadinol-note), spicy (mixture of spices used for ready-to-cook soups), vetiver-, osmanthus-, rose- (both β-phenylethyl alcohol-like) and lovage-notes (α-terpineol-like), weak tobacco side-note (root oil) and warm-herbal, fresh-medicinal, fruity-spice (like the root oil), vetiver-, osmanthus-, rose- and lovage-notes, tobacco side-note (sweet note when burned in a smoking-pipe) and aromatic-fatty notes in the background (herb oil).
Using gas chromatographic - spectroscopic systems more than 95 components could be identified (see Table 1) as genuine constituents of the three essential *S. indicus* oils. As main compounds (concentrations higher than 3.0%, calculated as %-peak-area of GC-FID analyses using an apolar column) of the essential oil of the flowers of *S. indicus* we found β-eudesmol (21.4%), 2,5-dimethoxy-p-cymene (16.2%), β-caryophyllene (7.8%), τ-cadinol (7.2%), caryophyllene oxide (6.9%) and α-eudesmol (4.5%). The root oil consists mainly of 2,5-dimethoxy-p-cymene (28.3%), τ-cadinol (25.3%), (*Z*)-arteannuic alcohol (10.1%), β-maaliene (3.9%) and caryophyllene oxide (3.1%) whereas in the herb oil 2,5-dimethoxy-p-cymene

(27.0%), τ-cadinol (12.5%), β-eudesmol (9.1%), α-eudesmol (7.0%) and caryophyllene oxide (4.7%) were found.

Table 1: Composition of the essential oils of the flowers (eof), roots (eor) and herb (eoh) of *Sphaeranthus indicus* from southern India.

Compounds[1]	eof[2]	eor	eoh	KI[3]
3-methyl butanol	nd[4]	tr[5]	0.1	733
2-methyl butanol	nd	nd	0.1	737
2-methyl but-3-enol	tr	nd	0.1	771
hexanal	0.3	0.1	0.2	778
(*E*)-2-hexenal	0.1	0.6	0.2	831
(*Z*)-3-hexenol	0.1	0.1	0.2	845
(*E*)-2-hexenol	0.1	tr	0.3	854
hexanol	0.2	1.3	1.5	859
heptanal	nd	tr	0.1	883
heptan-2-one	tr	nd	tr	902
α-thujene	0.1	0.1	0.3	944
α-pinene	0.2	0.1	0.2	951
β-pinene	0.1	0.1	0.1	957
heptanol	0.3	tr	0.1	960
1-octen-3-ol	0.1	0.4	0.2	964
6-methyl-5-hepten-2-one	0.2	tr	0.3	968
6-methyl-5-hepten-2-ol	0.8	nd	tr	979
(*E*)-3-hexenyl acetate	tr	0.1	0.4	982
myrcene	tr	tr	tr	985
(*E*)-2-hexenyl acetate	tr	tr	0.2	995
hexanoic acid	nd	tr	0.3	999
octanal	nd	0.2	0.1	1001
para-cymene	nd	0.1	0.1	1012
limonene	0.1	nd	0.1	1023
(*Z*)-β-ocimene	tr	0.2	tr	1027
(*E*)-β-ocimene	tr	0.1	tr	1033
(*Z*)-linalool oxide (furanoid)	0.2	nd	tr	1064
1-nonen-3-ol	0.1	nd	tr	1068
terpinolene	0.1	tr	0.3	1080
linalool	0.5	nd	0.2	1085
nonanal	0.2	tr	0.4	1087
β-phenylethyl alcohol	2.1	2.0	1.9	1107
borneol	2.0	tr	1.7	1169
terpinen-4-ol	0.4	tr	0.5	1174
verbenone	tr	0.1	0.9	1177
α-terpineol	0.1	0.8	0.3	1185
citronellol	0.2	nd	tr	1225
nerol	1.3	tr	1.1	1229

neral	0.2	nd	tr	1235
geraniol	2.6	0.2	1.0	1253
geranial	0.1	tr	tr	1265
7-epi-silphiperfol-5-ene	0.2	0.5	0.3	1348
eugenol	0.5	tr	2.0	1358
α-cubebene	tr	0.1	tr	1362
neryl acetate	0.2	0.1	0.6	1364
geranyl acetate	1.3	tr	0.6	1379
β-maaliene	1.1	3.9	2.9	1382
α-isocomene	0.3	0.8	0.4	1388
α-ylangene	tr	0.1	0.2	1394
α-copaene	tr	0.1	0.3	1399
β-cubebene	0.3	0.1	tr	1402
cyperene	0.1	nd	tr	1405
β-isocomene	0.3	0.6	0.4	1407
β-elemene	0.2	0.6	0.4	1410
α-gurjunene	0.1	0.3	0.1	1412
β-bourbonene	tr	0.2	0.1	1414
(*Z*)-α-bergamotene	0.3	0.4	0.1	1416
α-cedrene	nd	tr	0.4	1418
α-himachalene	0.1	nd	0.3	1420
β-caryophyllene	7.8	0.6	1.1	1424
aromadendrene	0.2	0.3	0.1	1427
γ-elemene	0.1	nd	tr	1429
2,5-dimethoxy-p-cymene	16.2	28.3	27.0	1431
(*E*)-α-bergamotene	0.3	0.4	tr	1435
(*E*)-β-farnesene	tr	nd	tr	1448
α-amorphene	0.1	0.2	0.1	1452
α-guaiene	0.4	0.1	0.5	1455
α-humulene	0.9	0.4	0.3	1462
germacrene D	tr	0.6	0.2	1468
alloaromadendrene	0.2	tr	0.2	1474
(*E*,*E*)-α-farnesene	tr	tr	tr	1490
α-muurolene	0.3	0.2	0.1	1494
(*Z*)-α-bisabolene	0.2	0.3	0.1	1497
δ-cadinene	0.3	0.2	0.6	1505
β-selinene	0.2	tr	0.1	1507
γ-cadinene	0.1	0.3	0.1	1510
(*Z*)-nerolidol	0.5	0.5	0.7	1536
elemol	0.6	0.9	0.4	1540
(*E*)-nerolidol	0.2	0.1	0.2	1551
spathulenol	0.4	0.6	0.3	1553
caryophyllene alcohol	0.5	0.7	0.4	1557
β-vetivene	0.2	0.8	0.7	1563
longifolene	0.1	0.3	0.1	1566
caryophyllene oxide	6.9	3.1	4.7	1574
α-cedrene epoxide	0.1	tr	0.6	1582
guaiol	0.2	0.4	0.4	1584
globulol	0.3	0.4	0.1	1587

(*Z*)-arteannuic alcohol[$]	1.2	10.1	0.4	1594
humulene oxide	1.6	1.2	0.3	1603
khusimone	tr	0.7	0.8	1605
cedrol	0.2	0.3	0.5	1609
(*E*)-arteannuic alcohol[$]	0.9	tr	nd	1616
γ-eudesmol	1.5	0.8	0.4	1631
β-eudesmol	21.4	1.0	9.1	1647
τ-cadinol	7.2	25.3	12.5	1649
α-cadinol	0.7	0.2	0.9	1652
α-eudesmol	4.5	0.3	7.0	1654
β-bisabolol	0.5	0.1	0.1	1663
α-guaiyl acetate	1.4	tr	1.0	1727
(*E*,*E*)-farnesol	0.1	tr	nd	1733
khusimol	0.1	0.5	0.6	1742
γ-eudesmyl acetate	0.6	0.4	0.3	1784
β-eudesmyl acetate	0.5	0.4	0.2	1792
α-eudesmyl acetate	1.1	0.2	2.0	1795
phytol	0.3	1.3	1.3	2080

[1]in order of their elution
[2]concentrations as %-peak-area calculated by GC-FID analysis
[3]Kovats indices from unpolar FSOT-RSL column
[4]not detected
[5]trace compound (less than 0.1%)
[$]identified in accordance to cited literature[17,18,24,25]

In accordance to these compositions, especially three points are remarkable: 1.) the relative high concentration of 2,5-dimethoxy-p-cymene in all three essential *Sphaeranthus indicus* oils from southern India. This aromatic monoterpene (syn. thymoquinol) was identified in e.g. oil samples of *Monarada* species, *Eupatorium japonicum*, *Juniperus chinensis* and *Libocedrus decurrens*[27], but not in a *Sphaeranthus* species until now. 2.) the sesquiterpenes α- and β-eudesmol, known target compounds of various *Eucalyptus* species[27], were found as free alcohols in such a significant concentration in *S. indicus* oils for the first time, while corresponding eudemanolides could be identified in different *S. indicus* extracts of previous works[4]. 3.) arteannuic alcohols were reported to be constituents of the essential oil of *Artemisia annua*[25] as well as of the essential oil of *Inula crithmoides*[24], but no data are available that these sesquiterpene alcohols with cadinane structure have been found in a *Sphaeranthus indicus* sample.

A comparison of analytical data with olfactoric ones, published elsewhere[28-32], allows following statements to the aroma impressions of essential flower, root and herb oils of *Sphaeranthus indicus* from southern India: warm-herbal aroma notes can be especially

attributed to 2,5-dimethoxy-p-cymene, dry-woody and spicy notes to cadinane and caryophyllane derivatives, fruity notes to minor aliphatic alcohols, such as hexanol, as well as some monoterpenes (e.g. geraniol), osmanthus- and rose-notes to 2-phenylethyl alcohol and the monoterpenes citronellol, geranial, geraniol, neral and nerol and lovage-like notes to α-terpineol, vetiver notes of β-vetivene, khusimone, khusimol and β-bourbonene.

An medicinal use of these essential *S. indicus* oils seems to be only possible as an antimicrobial medicament[33-37] for external application, because toxic effects and side-effects of the mainly sesquiterpenic constituents of the oils which are investigated very rarely until now. The same objection must be done on cosmetic applications as well as for the flavoring of food products.

In summary, the essential oils of the flowers, roots and herb of *Sphaeranthus indicus* from southern India possess a very characteristic odor, showing some uncommon monoterpenes, such as 2,5-dimethoxy-p-cymene, and sesquiterpenes, such as arteannuic alcohols and eudesmols as main compounds. These constituents as well as further lower alcohols and mono- and sesquiterpenes are responsible for the odor impressions. A possible use of these oils as medicaments, in cosmetic and/or food products can be discussed again, after extensive investigations of the toxic potential of these identified sesquiterpenic components.

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