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Chemical composition of the aliphatic compounds rich essential oil of *Hypericum japonicum* Thunb. ex Murray from India

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Hydro-distilled essential oil of the aerial parts of *Hypericum japonicum* Thunb. ex Murray (Hypericaceae), grown in northern India was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). A total of seventy constituents representing 93.6% of the total composition have been identified. Major constituents of the essential oil were 2-methyl octane (24.9%), *n*-nonane (21.4%), (2*Z*)-nonenol (16.5%), *n*-decanal (8.2%) and allo-aromadendrene epoxide (3.3%). The characteristic of the *H. japonicum* essential oil was the presence of higher amount of aliphatic compounds (82.5%) compared with terpenoids.

Keywords: *Hypericum japonicum*; Hypericaceae; essential oil composition; 2-methyl octane; *n*-nonane

Introduction

The genus *Hypericum* belongs to Hypericaceae (Clusiaceae) family and consists of 460 species, distributed chiefly in the temperate regions of the world and about twenty-five species occur in India (1, 2). Numerous *Hypericum* species are used as medicine by the inhabitants in one way or the other due to their therapeutic efficacy. Amongst the species, *Hypericum perforatum* is most popular today in different countries of the world especially as an antidepressant (3). Besides *H. perforatum*, other species of the genus are also economically important and are used as fodder, fuel, dye and for medicinal purposes (4).

Hypericum japonicum Thunb. ex Murray, commonly known as 'Pikarichar' in India, another member of the genus, is an annual decumbent or prostrate herb occasionally found growing near rice fields, ditches, marshes, grasslands and in waste lands from sea level up to 3000 m (1). Bioactive compounds, phloroglucinol derivatives sarothralen A, B, C, D and G, have been isolated from *H. japonicum*. The antimicrobial activities of these compounds were comparable with or greater than that of streptomycin against the Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and the acid-fast bacterium (*Mycobacterium smegmatis*) (5). The entire herb of *H. japonicum*, named 'Tianjihuang' in China, has been widely used for the treatment of bacterial diseases, infectious hepatitis, acute and chronic hepatitis, gastrointestinal disorder, internal hemorrhage and tumor. It has also been noted as hepatoprotective in rats (6–8).

Several studies have been published in recent years on essential oil composition of different *Hypericum* species from different parts of the world. The major constituents of the essential oils of genus *Hypericum* are aliphatic compounds and terpenoids (9–20).

A literature survey revealed that no attempt has been made to date to investigate the volatile phytomolecules of *H. japonicum* growing in India. Therefore, the present investigation reports a detailed gas chromatography (GC) and GC–mass spectrometry (GC–MS) profile of *H. japonicum* essential oil from Kumaon region of western Himalaya, India.

Experimental

Plant material

Hypericum japonicum was collected from wild (Purara, Bageshwar western Himalaya) during 2005 and transplanted in the experimental field of the Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara, for domestication. The plant was identified by botany department of Centre (Voucher Specimen: CIMPANT-294). The site is located at an altitude of 1250 m in the Kattyur valley, western Himalaya. Climatologically, it is categorized as a temperate zone. Fresh aerial parts of the *H. japonicum* were collected on 24 June 2009 at full flowering stage from a domesticated population.

Isolation of the essential oil

The essential oil of the fresh aerial parts of *H. japonicum* was extracted by hydrodistillation for 3 hours

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Table 1. Essential oil composition of *Hypericum japonicum* from Uttarakhand, India.

Compound	KI[a]	KI[b]	% of identified compound
(*E*)-Hex-2-enal	853	855	tr
2-Methyl octane	865	860	24.9
n-Nonane	904	900	21.4
α-Pinene	936	939	tr
Camphene	945	954	0.1
3-Methyl nonane	970	971	1.3
β-Pinene	980	979	tr
Myrcene	989	990	tr
Decane	1002	1000	0.1
α-Phellandrene	1004	1002	0.1
p-Cymene	1026	1024	0.5
Limonene	1030	1029	0.2
1,8-Cineole	1035	1031	tr
(*Z*)-*β*-Ocimene	1039	1037	tr
(*E*)-*β*-Ocimene	1048	1050	tr
γ-Terpinene	1060	1059	0.2
2-Methyl decane	1064	1067	0.4
(*E*)-2-Nonen-1-ol	1069	–	0.3
2-Nonanone	1091	1090	tr
n-Undecane	1102	1100	1.9
Nonanal	1105	1100	0.2
(2*Z*)-Nonenol	1172	1166	16.5
n-Nonanol	1177	1169	tr
α-Terpineol	1186	1188	tr
(4*Z*)-Decenal	1193	1194	0.1
n-Decanal	1208	1201	8.2
(3Z)-Hexenyl-2-methylbutanoate	1235	1232	tr
(3Z)-Hexenyl-3-methylbutanoate	1239	1235	3.0
3-Undecanone	1257	1255	0.1
n-Decanol	1270	1269	0.5
2-Undecanone	1294	1294	0.1
n-Tridecane	1304	1300	0.3
1,8-Octanediol	1346	1341	0.8
Decanoic acid	1373	1366	0.5
(2*E*)-Undecenol	1378	1367	0.1
β-Cubebene	1387	1388	tr
Sibirene	1396	1400	0.3
Dodecanal	1409	1408	1.3
α-Cedrene	1411	1411	tr
(*E*)-Caryophyllene	1421	1419	0.3
α-Aromadendrene	1438	1439	0.2
α-Humulene	1454	1454	0.5
(*E*)-*β*-Farnesene	1457	1456	tr
allo-Aromadendrene	1461	1460	tr
n-Dodecanol	1467	1470	0.3
10-*epi*-*β*-Acoradiene	1474	1475	0.4
Germacrene D	1485	1481	0.2
(*Z*)-Cadina-1,4-diene	1494	1495	0.1
10-Undecenol acetate	1498	1498	0.1
β-Himachalene	1502	1500	tr
(*E*,*E*)-*α*-Farnesene	1507	1505	0.1
δ-Amorphene	1511	512	tr
γ-Cadinene	1513	1513	tr
δ-Cadinene	1523	1523	tr
α-Calacorene	1543	1545	tr
(*E*)-Nerolidol	1564	1563	0.5
Spathulenol	1582	1578	tr
Caryophyllene oxide	1584	1583	0.1
Globulol	1591	1590	0.1
Humulene epoxide II	1610	1608	0.3

(*Continued*)

Table 1. (*Continued*).

Compound	KI[a]	KI[b]	% of identified compound
10-*epi*-γ-Eudesmol	1625	1623	tr
1-*epi*-Cubebol	1630	1628	0.2
γ-Eudesmol	1634	1632	0.2
allo-Aromadendrene epoxide	1641	1641	3.3
β-Eudesmol	1651	1650	tr
α-Cadinol	1656	1654	0.1
(*Z*)-α-Santalol	1673	1675	1.0
α-Bisabolol	1685	1685	1.0
(2*Z*,6*E*)-Farnesol	1722	1723	1.1
Octadecane	1800	1800	0.1
Grouped components			
Aliphatics			82.5
Terpenoids			11.1
Monoterpene hydrocarbons			1.1
Sesquiterpene hydrocarbons			2.1
Oxygenated monoterpenes			tr
Oxygenated sesquiterpene			7.9
Identified compounds			93.6

Notes: KI[a], Kovat's Index (experimental) on DB-5 column relative to *n*-alkane (C_8–C_{25}); KI[b], Kovat's Index literature (Adams, 2007); tr: trace (component <0.05%).

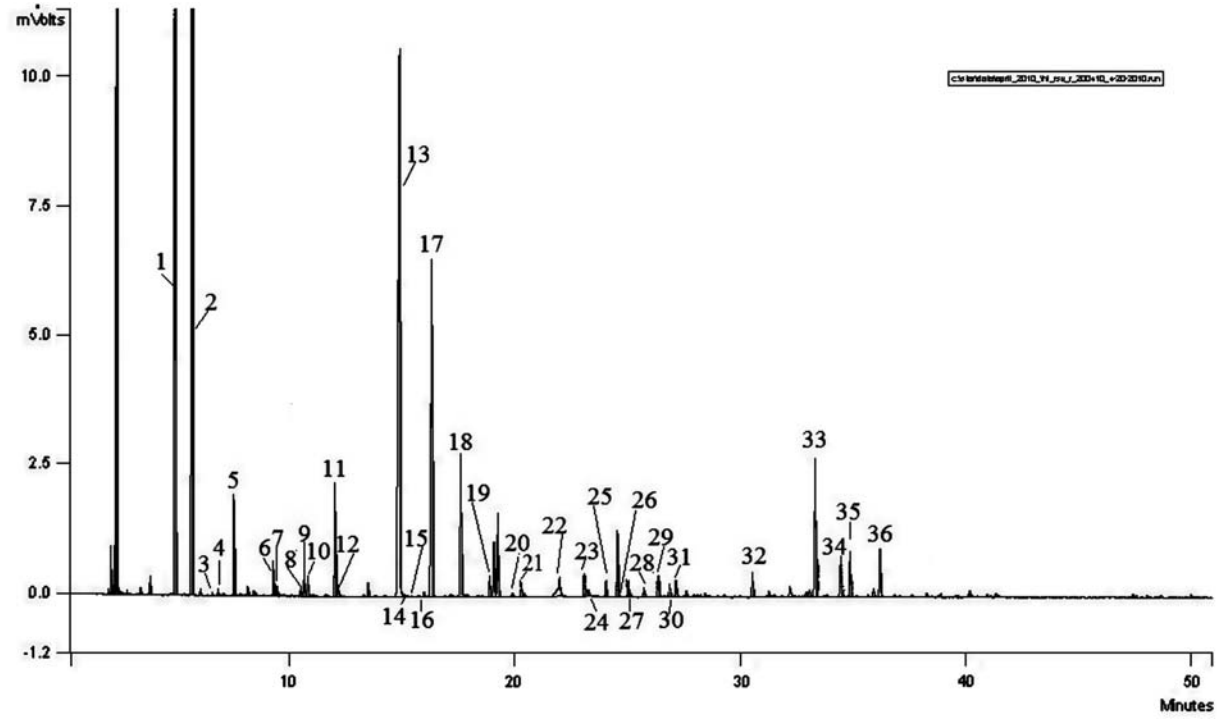

Figure 1. Chromatogram (gas chromatography/flame ionization detector) of *Hypericum japonicum* essential oil from India (Peaks; **1**: 2-methyl octane, **2**: *n*-nonane, **3**: α-pinene, **4**: camphene, **5**: 3-methyl nonane, **6**: *p*-cymene, **7**: limonene, **8**: γ-terpinene, **9**: 2-methyl decane, **10**: (*E*)-2-nonen-1-ol, **11**: *n*-undecane, **12**: nonanal, **13**: (2*Z*)-nonenol, **14**: *n*-nonanol, **15**: α-terpineol, **16**: (4*Z*)-decenal, **17**: *n*-decanal, **18**: (3Z)-hexenyl-3-methylbutanoate, **19**: *n*-decanol, **20**: 2-undecanone, **21**: *n*-tridecane, **22**: 1,8-octanediol, **23**: decanoic acid, **24**: (2*E*)-undecenol, **25**: sibirene, **26**: dodecanal, **27**: (*E*)-caryophyllene, **28**: α-aromadendrene, **29**: α-humulene, **30**: *n*-dodecanol, **31**: 10-*epi*-β-acoradiene, **32**: (*E*)-nerolidol, **33**: allo-aromadendrene epoxide, **34**: (*Z*)-α-santalol, **35**: α-bisabolol, **36**: (2*Z*,6*E*)-farnesol). For other minor/trace constituents, please see Table 1.

using a Clevenger apparatus (21). The percentage essential oil content (%v/w) was estimated on a fresh weight basis. The oil sample obtained was dehydrated over anhydrous sodium sulfate and kept in a cool and dark place before analyses.

Gas chromatography

The GC analysis of the oil samples was carried out on Perkin–Elmer Auto XL GC, fitted with an Equity-5 column (60 m × 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven column temperature ranged from 70 to 250°C, programmed at 3°C/minute, with initial and final hold time of 2 minutes, using H_2 as the carrier gas at 1.0 mL/minute, a split ratio of 1:30, an injection size of 0.03 µL neat, and injector and detector (FID) temperatures were 250 and 280°C, respectively.

Gas chromatography/mass spectrometry

GC–MS analysis of the essential oil sample was carried out on a Perkin–Elmer AutoSystem XL GC interfaced with a Turbomass Quadrupole mass spectrometer fitted with an Equity-5 fused-silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm; Supelco Bellefonte, PA, USA). The oven temperature program was the same as described in capillary GC; injector, transfer line and source temperatures were 250°C; injection size 0.03 µL neat; split ratio 1:30; carrier gas He at 1.0 mL/minute; ionization energy 70 eV; mass scan range 40–450 amu.

Identification of compounds

Identification of the essential oil constituents was done on the basis of retention time, and Kovat index, using a homologous series of *n*-alkanes (C_8–C_{25} hydrocarbons, Polyscience Corp., Niles, IL, USA) under identical experimental conditions, co-injection with standards (Aldrich and Fluka) or known essential oil constituents, mass spectra library search (NIST/EPA/NIH version 2.1 and *Wiley Registry of Mass Spectral Data*, 7th edition) and by comparing the mass spectral and retention data with literature (22). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Results and discussion

The essential oil yield in fresh aerial parts of *H. japonicum* was 0.22% (±0.01). The resulted essential oil was analyzed by GC and GC–MS. A total of seventy constituents, forming 93.6% of the total oil composition were identified (Table 1). A chromatogram (GC/FID) of the essential oil is shown in Figure 1. The oil was dominated by aliphatic compounds (82.5%) followed by oxygenated sesquiterpenes (7.9%) and sesquiterpene hydrocarbons (2.1%). Major aliphatic components of this essential oil were 2-methyl octane (24.9%), *n*-nonane (21.4%), (2*Z*)-nonenol (16.5%), *n*-decanal (8.2%), (3*Z*)-hexenyl-3-methylbutanoate (3.0%), *n*-undecane (1.9%), 3-methyl nonane (1.3%), dodecanal (1.3%) and 1,8-octanediol (0.8%). Oxygenated sesquiterpenes noted in higher amount in this essential oil were allo-aromadendrene epoxide (3.3%), (2*Z*,6*E*)-farnesol (1.1%), (*Z*)-α-santalol (1.0%), α-bisabolol (1.0%) and (*E*)-nerolidol (0.5%). Further, representative sesquiterpene hydrocarbons of the oil were α-humulene (0.5%), 10-*epi*-β-acoradiene (0.4%) and (*E*)-caryophyllene (0.3%).

Essential oil composition of the different *Hypericum* species has been investigated earlier from different parts of the world. In general, all the investigated taxa of genus *Hypericum* are characterized by the presence of higher amount of aliphatics or terpenoids. The species like *H. hirsutum*, *H. caprifoliatum*, *H. foliosum*, *H. hircinum* and *H. undulatum* are dominated by aliphatic compounds; however, *H. perforatum*, *H. alpinum*, *H. barbatum*, *H. rumeliacum*, *H. maculatum* and so on are characterized by the presence of components of terpenoids group (2, 9, 12, 23). So far, essential oil composition of only one *Hypericum* species (*H. perforatum*) has been studied from India and it belongs to terpenoids (α-pinene) group (10). However, *H. japonicum*, investigated in present study, showed similarity to *Hypericum* species rich in aliphatic compounds. The antimicrobial activity of the *Hypericum* essential oils is not attributed to aliphatic hydrocarbons (alkanes), because of their limited hydrogen capacity and water solubility (20, 25). However, the *H. japonicum* is being used for the treatment of several bacterial diseases, infectious hepatitis, gastrointestinal disorder and tumors (26). These activities are might be due to non-volatile compounds (xanthones, chromenes, flavanonols, dipeptide derivatives and phloroglucinol derivatives) present in *H. japonicum* (26).

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