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Essential oil composition of *Sphagneticola trilobata* (L.) Pruski from India

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This study aimed to explore the potential of an invasive alien species, *Sphagneticola trilobata* (L.) Pruski, that grows in foothill region of northern India. The volatile oil from the aerial parts of *S. trilobata* was isolated by hydrodistillation method and analysed using capillary gas chromatography–flame ionization detector (GC–FID) and GC–mass spectrometry (GC–MS) during different seasons. Volatile oil yield varied from 0.18 to 0.25% in different seasons, with the maximum in winter season. Altogether, 43 constituents, representing 96.1–97.3% of the total oil composition were identified. Major constituents of the oils were α-pinene (78.6–83.3%), α-phellandrene (1.3–4.1%), sabinene (1.4–1.9%), limonene (1.2–1.9%), β-pinene (1.0–1.6%), camphene (0.7–2.0%), 10-nor-calamenen-10-one (<0.05–1.5%), germacrene D (0.1–1.4%) and γ-amorphene (<0.05–1.3%). The comparative results showed no big differences in the oil composition of this plant due to season of collection. It is concluded that the *S. trilobata* population grown in this region could be utilized as a potential source of industrial molecule, α-pinene.

Keywords: *Sphagneticola trilobata*; essential oil composition; seasonal variation; α-pinene

1. Introduction

Sphagneticola trilobata (L.) Pruski, previously known as *Wedelia trilobata* (L.) Hitchc., belongs to family Asteraceae and naturally grows in coastal regions, barren lands and forests, in many countries (1). It is a creeping evergreen perennial herb, spreading widely and preventing regeneration of other species. It has a very wide ecological tolerance range and can tolerate inundation and high levels of salinity (2). This species has several synonyms and the most common in Brazil is *Wedelia paludosa* DC (3). In Australia, it is known as Singapore Daisy and in some other countries it is known as *Wedelia*, trailing or creeping daisy, water zinnia and rabbit's paw (1). *S. trilobata* is employed to treat backache, muscle cramps, rheumatism, stubborn wounds, sores and swellings, and arthritic painful joints (4). Leaves are reported to be used in the treatment of kidney problems, cold, wounds, snakebite and amenorrhoea (5). *S. trilobata* has been reported to have antibacterial (6), anticonceptive (7), trypanosomicidal (8) and anti-inflammatory (9) activities.

It is well known that climatic conditions and water available in the soil can change the vegetal secondary metabolism and, consequently, alter the composition of essential oils throughout the seasons of the year (3, 10–13). A literature survey revealed that chemical composition of the essential oil of *S. trilobata* has been investigated earlier (3, 14–17), but no attempt was made previously to explore the chemical compositions of essential oil of *S. trilobata* collected in different seasons from northern India. The present study was, therefore, planned to analyse and compare, the essential oil yield and composition of *S. trilobata* population (α-pinene chemotype) collected in summer, rainy, autumn and winter seasons from foothills of northern India.

2 Experimental

2.1 Plant material

Fresh aerial parts of the *S. trilobata* were collected from CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar (Uttarakhand) in summer (vegetative stage), rainy (vegetative stage), autumn (flowering stage) and winter (flowering stage) seasons. The experimental site is located between coordinates 29.02° N, 79.31° E and an altitude of 243 m in foothills of northern India. The plant material was authenticated at botany department of the centre (voucher specimen number: CIMPANT-346) by one of the author (AC).

2.2 Isolation of essential oil

Isolation of the essential oil from fresh plant material was carried out by hydrodistillation in a Clevenger-type apparatus for three hours. Isolated oils were dried over anhydrous Na_2SO_4 and stored at 4°C until analysis.

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2.3 Essential oil analysis

GC analysis of the essential oil was carried out on a Nucon gas chromatograph model 5765 equipped with DB-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) or on a PerkinElmer AutoSystem XL gas chromatograph equipped with DB-5 capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm) and flame ionization detector (FID). The oven column temperature ranged from 60–230°C, programmed at 3°C/min using H_2 as carrier gas at 10 psi constant pressure, a split ratio

Table 1. Essential oil composition of *Sphagneticola trilobata* (L.) Pruski collected during different seasons.

		% Relative peak area				
RI	Compound	Summer	Rainy	Autumn	Winter	Mean ± SD
928	α-Thujene	*t*	0.3	0.1	0.1	0.13 ± 0.12
936	α-Pinene	83.3	78.6	82.1	82.5	81.63 ± 2.08
945	Camphene	2.0	1.0	0.7	0.8	1.13 ± 0.60
952	Thuja-2,4(10)-diene	0.1	*t*	*t*	*t*	0.05 ± 0.04
970	Sabinene	1.8	1.9	1.8	1.4	1.73 ± 0.22
974	β-Pinene	1.6	1.3	1.3	1.0	1.30 ± 0.24
991	Myrcene	0.1	*t*	0.2	*t*	0.09 ± 0.08
995	Unidentified[†]	0.5	1.0	0.5	0.6	0.65 ± 0.24
1003	α-Phellandrene	1.3	3.5	3.1	4.1	3.00 ± 1.21
1023	*p*-Cymene	0.8	0.6	0.5	0.3	0.55 ± 0.21
1026	Limonene	1.2	1.4	1.5	1.9	1.50 ± 0.29
1033	(*Z*)-β-Ocimene	0.1	0.2	0.2	0.7	0.30 ± 0.27
1044	(*E*)-β-Ocimene	0.2	0.2	0.1	0.1	0.15 ± 0.06
1062	Acetophenone	0.1	0.2	*t*	0.1	0.11 ± 0.07
1078	*m*-Cymenene	*t*	–	*t*	–	0.02 ± 0.02
1086	Fenchone	–	0.1	*t*	–	0.03 ± 0.05
1106	1,3,8-*p*-Methatriene	*t*	0.1	*t*	*t*	0.05 ± 0.04
1117	Unidentified[††]	*t*	*t*	*t*	*t*	0.03 ± 0.01
1125	α-Campholenal	0.1	*t*	*t*	*t*	0.05 ± 0.04
1138	*trans*-Pinocarveol	*t*	*t*	*t*	–	0.02 ± 0.02
1143	*trans*-Sabinol	0.1	0.1	*t*	*t*	0.07 ± 0.04
1163	Pinocarvone	*t*	–	*t*	–	0.02 ± 0.02
1168	Borneol	0.1	–	*t*	*t*	0.04 ± 0.04
1177	Terpenen-4-ol	1.2	0.2	*t*	–	0.36 ± 0.57
1186	*p*-Cymen-8-ol	*t*	–	*t*	–	0.02 ± 0.02
1189	Cryptone	*t*	–	*t*	–	0.02 ± 0.02
1192	α-Terpineol	*t*	*t*	0.1	–	0.04 ± 0.04
1197	Myrtenol	0.1	*t*	*t*	–	0.04 ± 0.04
1200	Myrtenal	0.1	*t*	*t*	*t*	0.05 ± 0.04
1290	Thymol	*t*	–	*t*	*t*	0.02 ± 0.02
1351	α-Cubebene	*t*	0.1	*t*	*t*	0.05 ± 0.04
1377	α-Copaene	0.1	*t*	*t*	*t*	0.05 ± 0.04
1415	Unidentified[†††]	0.2	–	*t*	*t*	0.07 ± 0.09
1417	(*E*)-Caryophyllene	0.5	0.6	0.7	0.1	0.48 ± 0.26
1454	α-Humulene	0.1	0.1	*t*	–	0.06 ± 0.05
1478	γ-Gurjunene	0.2	0.1	0.1	*t*	0.09 ± 0.08
1482	Germacrene D	0.1	0.6	1.1	1.4	0.80 ± 0.57
1496	γ-Amorphene	*t*	1.1	1.3	1.1	0.88 ± 0.58
1499	α-Muurolene	0.3	0.1	*t*	0.1	0.13 ± 0.12
1522	δ-Cadinene	*t*	0.4	0.3	0.2	0.23 ± 0.16
1565	*(E)*-Nerolidol	0.1	*t*	*t*	–	0.04 ± 0.04
1571	Longipinanol	*t*	0.1	*t*	*t*	0.05 ± 0.04
1579	Spathulenol	0.2	0.4	0.1	*t*	0.18 ± 0.16
1582	Caryophyllene oxide	0.1	0.2	0.3	0.3	0.23 ± 0.10
1625	1-epi-Cubebol	0.1	0.3	*t*	0.4	0.21 ± 0.17
1697	10-nor-Calamenen-10-one	0.2	1.5	*t*	0.1	0.46 ± 0.70
	Total identified (%)	97.0	96.3	96.1	97.3	96.68 ± 0.57
	Essential oil yield (%)*	0.18	0.19	0.22	0.25	0.21 ± 0.03

Notes: RI; retention index calculated on DB-5 capillary column; t; trace (<0.05%).
[†]m/e; 44(100), 51(30), 65(28), 77(45), 91(93), 119(41), 134(26).
[††]m/e; 43(53), 44(55), 51(40), 53(35), 77(50), 79(50), 91(100), 107(28), 115(66), 117(90), 132(38).
[†††]m/e; 41(100), 43(98), 55(40), 69(50), 91(43), 98(43), 135 (70), 150(20), 168(3); *Fresh weight basis.

of 1:35, an injection size of 0.03 µL neat and injector and detector temperatures were 220 and 230°C, respectively, for Nucon gas chromatograph model 5765. The oven column temperature ranged from 70–250°C, programmed at 3°C/min, with initial and final hold time of 2 minutes using H_2 as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03 µL neat and injector and detector temperatures were 250 and 280°C, respectively, for PerkinElmer AutoSystem XL gas chromatograph.

GC–MS analysis was carried out on a PerkinElmer 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). The oven temperature programme was from 70 to 250°C at 3°C/min with initial and final hold time of 2 minutes; injector, transfer line and source temperatures were 250°C; injection size 0.03 µL neat; split ratio 1:30; carrier gas He at 10 psi constant pressure; ionization energy 70 eV; and mass scan range 40–450 amu.

Characterization was achieved on the basis of retention time and retention index using a homologous series of *n*-alkanes (C_8–C_{30} hydrocarbons), co-injection with standards in GC–FID capillary column (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data (18). The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

3 Results and discussions

The essential oil yield in fresh aerial parts of *S. trilobata* was ranged between 0.18 and 0.25% in different seasons. Yield was found to be higher in winter season (0.25%) followed by autumn season (0.22%). Earlier, essential oil yield in fresh leaves of *S. trilobata* was found to be 0.10% (14). Further, Silva et al. (3) also investigated essential oil of *S. trilobata* from Brazil and reported highest essential oil yield in the plant material collected during winter season (3). Essential oils of *S. trilobata* obtained from different seasons were analysed by GC–FID and GC–MS. A total of 43 constituents representing 96.1–97.3% of the total oil compositions were identified (Table 1). Major constituents of the oils were *α*-pinene (78.6–83.3%), *α*-phellandrene (1.3–4.1%), sabinene (1.4–1.9%), limonene (1.2–1.9%), *β*-pinene (1.0–1.6%), camphene (0.7–2.0%), 10-nor-calamenen-10-one (<0.05–1.5%), germacrene D (0.1–1.4%), *γ*-amorphene (<0.05–1.3%), *p*-cymene (0.3–0.8%), (*Z*)-*β*-ocimene (0.1–0.7%) and (*E*)-caryophyllene (0.1–0.7%). GC–FID chromatogram of the *S. trilobata* essential oil with labelled major peaks is shown in Figure 1. Although, there were no considerable changes noticed in the oil composition of *S. trilobata* collected in different seasons, *α*-pinene was observed to be slightly lower and sabinene, *δ*-cadinene and 10-nor-calamenen-10-one slightly higher during the rainy season as compared to other seasons. Moreover, camphene, *β*-pinene, *p*-cymene and terpinen-4-ol were recorded relatively higher in summer; *γ*-amorphene and (*E*)-caryophyllene reached their higher values

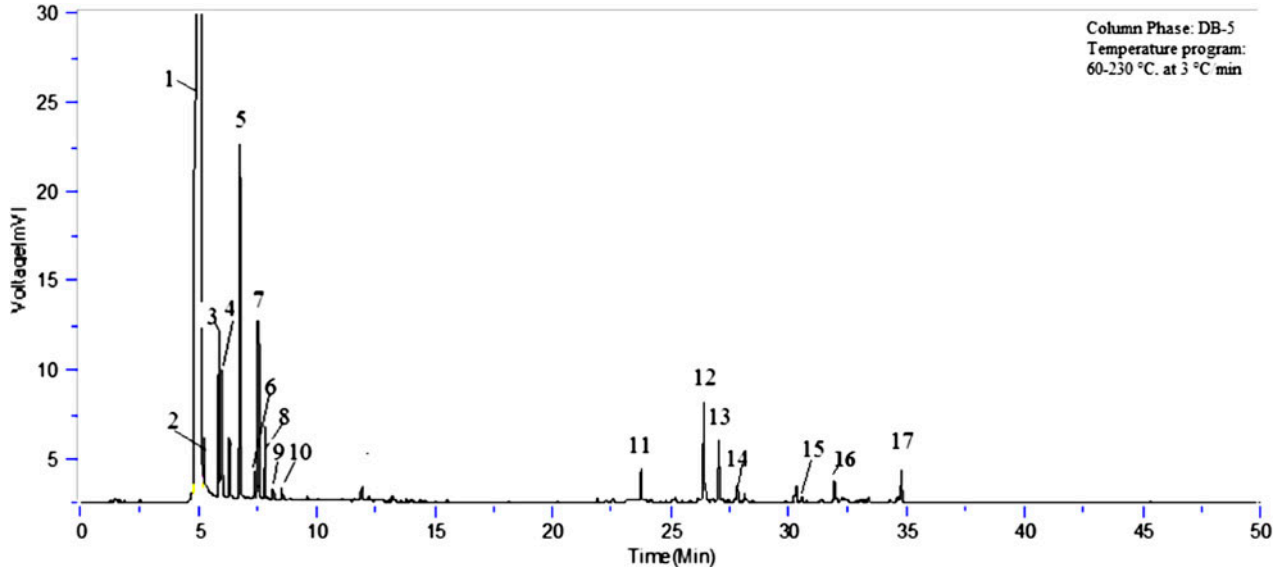

Figure 1. Chromatogram (GC–FID) of *Sphagneticola trilobata* essential oil from winter season (Peaks; 1: *α*-pinene, 2: camphene, 3: sabinene 4: *β*-pinene, 5: *α*-phellandrene, 6: *p*-cymene, 7: limonene, 8: (*Z*)-*β*-ocimene, 9: (*E*)-*β*-ocimene, 10: acetophenone, 11: (*E*)-caryophyllene, 12: germacrene D, 13: *γ*-amorphene; 14: *δ*-cadinene; 15: caryophyllene oxide; 16: 1-epi-cubebol; 17: 10-nor-calamenen-10-one.

in autumn; while the α-phellandrene, limonene, (*Z*)-β-ocimene and germacrene D were found to be more abundant in winter season than the other seasons.

Earlier, essential oil composition of *S. trilobata* has been investigated from other countries. Oil composition reported from China contained α-phellandrene (28.8%), germacrene D (15.7%) and limonene (14.2%) as main constituents (19). However, Silva et al. (3) found germacrene D (11.9–35.8%), α-phellandrene (1.4–28.5%), α-pinene (7.3–23.8%), (*E*)-caryophyllene (4.6–19.0%), bicyclogermacrene (6.0–17.0%), limonene (1.8–15.1%) and α-humulene (4.0–11.6%) as main components of *S. trilobata* population growing in Brazil (3). Conversely, according to another study from Brazil, α-pinene (30.3%), α-phellandrene (17.4%), β-pinene (6.4%), limonene (16.3%) and γ-muurolene (5.3%) were the principal constituents of the volatile oil this plant (14). Thus, the essential oil compositions of this plant reported earlier from different places differed considerably. These variations are probably a reflection of the geographical differences (12) or the chemotypic differences in the populations (20). Moreover, the present plant species would be a commercially viable source of α-pinene because α-pinene remains high at all collection times. The stability in chemical composition of *S. trilobata* most probably has a genetic, not an environmental basis.

In the light of seasonal variation study, the *S. trilobata* population growing in northern region of India could be classified as seasonally stable 'α-pinene' chemotype. Therefore, this invasive alien species could be exploited as a potential source of α-pinene, which possess anti-malarial activity (21) and used as an important substance in the manufacture of a variety of synthetic aroma chemicals and its epoxide is isomerized to produce campholenic aldehyde, which is an intermediate for the sandalwood fragrance, santalol (22).

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