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Volatile Constituents of Three Invasive Weeds of Himalayan Region

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Abstract: The volatile constituents of three most common aromatic exotic weeds viz. *Lantana camara* L., *Eupatorium adenophorum* Spreng and *Ageratum conyzoides* L. of the Uttarakhand, India were analyzed by GC and GC-MS. The essential oil of *Lantana camara* L. was dominated by sesquiterpenoids (70.8%) represented by sesquiterpene hydrocarbons (68.7%) with germacrene D (27.9%), germacrene B (16.3%), β-caryophyllene (9.6%), β-selinene (6.2%), α-humulene (5.8%) as major constituents. Other constituents in significant amount were sabinene (5.6%) and 1,8-cineole (4.8%). Amorphenes viz. amorph-4-en-7-ol (9.6%), 3-acetoxyamorpha-4,7(11)-dien-8-one (7.8%) and amorph-4,7(11)-dien-8-one (5.7%) were identified as the marker constituents of *Eupatorium adenophorum* Spreng along with p-cymene (16.6%), bornyl acetate (15.6%) and camphene (8.9%). On the contrary, the essential oil of *Ageratum conyzoides* L. was characterized by the presence of high percentages of ageratochromene (precocene II, 42.5%), β-caryophyllene (20.7%), demethoxyageratochromene (precocene I, 16.7%), α-humulene (6.6%) and p-cymene (3.3%).

Keywords: *Lantana camara; Ageratum conyzoides; Eupatorium adenophorum;* germacrene D; precocene; amorphene; essential oils.

1. Introduction

The Himalayan region is known to be a repository of important medicinal and economic plants since time immemorial. Invasion of exotic weeds threaten ecosystems, habitats or species with economic/environmental consequences [1-4]. These are non-native or exotic organisms that occur outside their natural adapted ranges and having wide dispersal potential. *Lantana camara* L. (Verbenaceae), *Ageratum conyzoides* L. (Asteraceae) and *Eupatorium adenophorum* Spreng

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(Asteraceae) are very common weeds widely found in the Himalayan region. Invasion of these exotic weeds have replaced the larger part of the vegetation in Uttarakhand and are considered as major threat to native plants and animals [5-6]. Despite their obnoxious nature and bad economic/environmental consequences these are reported to possess diverse medicinal properties and finds use in traditional medicines [7-13]. *Eupatorium adenophorum* used in folk medicines as antimicrobial, antiseptic, blood coagulant, analgesic, antipyretic and enhancer of phenobarbitone induced sleep [7-9]. The essential oil from *Lantana camara, Eupatorium adenophorum* and *Ageratum conyzoides* also exploited as insecticide and nematicide. Both *Lantana* and *Ageratum* are widely utilized for the treatment of colic pain, colds and fevers, diarrhea, rheumatism, spasms, and as a tonic [10-13]. But care must be taken for their internal use due to toxicity issues. They can cause liver lesions and tumors, nausea and other toxic consequences [14-16].

Literature survey revealed that the essential oil composition of *Lantana camara* L., *Eupatorium adenophorum* Spreng and *Ageratum conyzoides* L. have been investigated earlier from different countries which report variety of terpenoid and other biologically active constituents from these plants [17-33]. However, information from India is very meager and there is no report on the terpenoid composition of these aromatic weeds from Himalayan region. This prompted us to carry out detailed chemical investigation of *Lantana camara* L., *Eupatorium adenophorum* Spreng and *Ageratum conyzoides* L. from Kumaun region of Himalaya (Uttarakhand, India) for their possible utilization and having an overview on their aroma profile.

2. Materials and Methods

2.1. Plant Material

The botanical material (fresh flowering aerial part) of *Lantana camara* L., *Eupatorium adenophorum* Spreng and *Ageratum conyzoides* L. were collected from different habitat of Kumaun Himalaya. Plant herbarium and voucher specimens have been deposited and maintained in CIMAP Research Center, Pantnagar, Uttarakhand (India).

2.2 Isolation of Essential Oils

The fresh flowering aerial parts (1 kg each) were subjected to hydro-distillation using Clevenger-type apparatus for 3 hours. The oils were dried over anhydrous Na_2SO_4 and were stored in sealed vials under refrigeration prior to analysis. The oil yield was 0.40% in *Lantana camara*, 0.56% in *Eupatorium adenophorum* and 0.30% in *Ageratum conyzoides*.

2.3 Gas Chromatography GC)

The GC analysis of the oil samples was carried out on Nucon 5765 gas chromatograph equipped with dual FID, using two different stationary phases Rtx-5 (30 m × 0.32 mm i.d., 0.25 µm film coating) and CP Wax 52 CB (30 m × 0.32 i.d., 0.25 µm film thickness) fused silica columns, respectively. Nitrogen and Hydrogen were the carrier gas at 1.0 ml / min in nonpolar and polar column respectively. Temperature programming was from 70^0C-250^0C at 3^0C/min (CP Sil-8 CB) and from

60^0C-210^0C at 4^0C/min (Rtx-5) respectively. The injector and detector temperatures were 210°C and 220°C, respectively. The injection volume was 0.02 µL neat and 0.1 µL in hexane, split ratio was 1: 30.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the oil was carried out on Finnigan MAT PolarisQ ion trap mass spectrometer fitted with Rtx-5 fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating). The column temperature was programmed from 60^oC-210^oC at 3^oC /min using He as carrier gas at 1.0 mL/min. The injector temperature was 210^oC, injection size 0.1µL prepared in *n*-hexane, split ratio 1:40. MS were taken at 70 eV with mass scan range of 40-450 amu. Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of n-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL) under identical experimental condition), co injection with standards (Sigma and standard isolates), MS Library search (NIST and WILEY), by comparing with the MS literature data [34]. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

3. Results and Discussion

The identified constituents of the oils are listed in Table 1. A total of 44 compounds were identified accounting for 90.3% to 94.6% of the oils. The essential oil of *Lantana camara* L. was dominated by sesquiterpenoids (70.8%) represented by sesquiterpene hydrocarbons (68.7%) with germacrene D (27.9%), germacrene B (16.3%), β-caryophyllene (9.6%), β-selinene (6.2%), α-humulene (5.8%) as major constituents. Other constituents in significant amount were monoterpenoids (19.5%) with sabinene (5.6%) and 1,8-cineole (4.8%) as the representative constituents. Amorphenes (24.0%) were identified as the significant marker constituents of *Eupatorium adenophorum* along with p-cymene (16.6%), bornyl acetate (15.6%), and camphene (8.9%). The amorphene derivatives identified in *Eupatorium adenophorum* were amorph-4-en-7-ol (9.6%), 3-acetoxyamorpha-4,7(11)-dien-8-one (7.8%) and amorph-4,7(11)-dien-8-one (5.7%). Previously reported biologically active sesquiterpene lactones, cadinenes, chromenes and thymol derivatives were not detected in present analysis except for the presence of methyl thymol as a trace (<0.1%) constituent [17-20, 35-36]. The essential oil of *Ageratum conyzoides* was characterized by the presence of high percentages of chromene derivatives (59.2%) viz. ageratochromene (precocene II, 42.5%), β-caryophyllene (20.7%), demethoxyageratochromene (precocene I, 16.7%) along with α-humulene (6.6%) and p-cymene (3.3%) as major constituents. This is for the first time the comparative essential oil compositions of these three aromatic weeds have been reported from the Kumaun Himalaya, Uttarakhand, India.

Table 1. Essential oil composition of *L. camara, E. adenophorum* and *A. conyzoides*

Compounds	RI	Contents (FID %)			Mode of identification
		Lantana camara	*Eupatorium*† *adenophorum*	*Ageratum conyzoides*	
α-thujene	930	t	0.3	-	RI, MS
α-pinene	939	0.9	0.2	t	RI, MS
camphene	952	0.2	8.9	0.1	RI, MS
sabinene	980	5.6	-	0.2	RI, MS
β-pinene	982	0.9	t	t	RI, MS
Δ-2-carene	1002	-	0.3	-	RI, MS
α-phellandrene	1005	0.2	4.5	-	RI, MS
p-cymene	1026	t	16.6	3.3	RI, MS
limonene	1032	-	0.2	-	RI, MS
1,8-cineole	1033	4.8	-	t	RI, MS, Peak enrichment
(*E*)-β-ocimene	1052	0.5	-	0.1	RI, MS
terpinolene	1089	0.1	0.6	t	RI, MS
linalool	1098	2.0	t	0.2	RI, MS
camphor	1144	-	0.2	-	RI, MS
borneol	1165	0.8	0.4	0.1	RI, MS
p-menth-1,5-dien-8-ol	1168	-	0.9	-	RI, MS
methyl thymol	1234	-	t	-	RI, MS
methyl carvacrol	1245	-	0.4	-	RI, MS
bornyl acetate	1285	3.5	15.6	t	RI, MS, Peak enrichment
trans-pinocarvyl acetate	1297	-	0.4	-	RI, MS
neryl acetate	1366	-	0.7	-	RI, MS
Isoitalicene	1399	-	0.6	-	RI, MS
β-caryophyllene	1418	9.6	0.8	20.7	RI, MS, Peak enrichment
trans-α-bergamotene	1438	-	0.7	-	RI, MS
α-humulene	1452	5.8	0.3	6.6	RI, MS
(E)-β-farnesene	1458	2.9	t	t	RI, MS
demethoxyageratochromene (precocene I)	1460	-	-	16.7	RI, MS
amorpha-4,7(11)-diene	1464	-	0.3	-	RI, MS
germacrene D	1480	27.9	1.9	1.0	RI, MS, Peak enrichment
β-selinene	1485	6.2	0.6	-	RI, MS
α-bulnesene	1505	-	2.8	-	RI, MS
germacrene B	1535	16.3	-	-	RI, MS
selina-3,7(11)-diene	1544	-	1.1	-	RI, MS
germacren D-4ol	1575	1.2	2.1	0.2	RI, MS
Spathulenol	1578	0.5	1.1	t	RI, MS
epi-cubenol	1626	-	1.6	-	RI, MS
amorph-4-en-7-ol	1628	-	9.6	-	RI, MS
epi-α-cadinol	1642	t	0.4	t	RI, MS
ageratochromene (precocene II)	1654	-	-	42.5	RI, MS
α-cadinol	1656	0.4	6.2	0.1	RI, MS
amorph-4,7(11)-dien-8-one	1702	-	5.7	-	RI, MS
amorph-4-en-3,8-dione	1726	-	0.6	-	RI, MS
muurol-4-en-3,8-dione	1758	-	0.2	-	RI, MS
3-acetoxyamorpha-4,7(11)-dien-8-one	1792	-	7.8	-	RI, MS
Total identified (%)		**90.3**	**94.6**	**91.8**	

†Part of our previous results on oil composition of *Eupatorium adenophorum* Spreng [33]

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