Variation in the volatile phytochemistry of *Ageratum conyzoides*

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Abstract: *Ageratum conyzoides*, billy-goat weed, is an introduced invasive weed species to Asia, but is used in traditional herbal medicine in Nepal. The essential oil from the aerial parts of *A. conyzoides* growing wild in Biratnagar, Nepal, was obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry. A total of forty compounds were identified representing 99.7% of the oil composition. The major components were precocene I (61.7%) and precocene II (23.5%). The essential oil was screened for antimicrobial activity, nematicidal activity, and brine shrimp lethality, but was largely inactive in these bioassays. A hierarchical cluster analysis based on the compositions of this essential oil composition and compositions reported in the literature revealed two distinct clusters based on the relative concentrations of precocene I and precocene II.
Key-words: Essential oil composition, Ageratochromene, Precocene, Cluster analysis

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1. Introduction

There are some 40 species of *Ageratum* in the Asteraceae, all of which are native to the Neotropics [1]. *Ageratum conyzoides* L. (billy-goat weed) is native to South America, but it is now distributed throughout the tropics. In many locations it is a noxious invasive weed, including sub-Saharan Africa [2–4], China [5], India [6,7], Bangladesh [8], and Nepal [9]. Several reviews of *A. conyzoides* have appeared summarizing the phytochemistry, ethnopharmacological uses, and biological activities of the plant [10–12]. Tribal people in the Seti River area of western Nepal apply the leaf juice of *A. conyzoides* to cuts and wounds [13]. People from Kumoun, Uttrakhand, India use the leaf extract to stop bleeding [14] and to treat skin diseases (ringworm, scabies, sores, burns boils, cuts) [15]. In this report, we present the chemical composition of *A. conyzoides* essential oil collected from Biratnagar, Nepal, and analyze the chemical differences of *A. conyzoides* essential oils from other geographical locations.

2. Materials and Methods

2.1 Plant Material

Ageratum conyzoides was collected from city of Biratnagar (26°28′N, 87°16′E, 72 m above sea level) in Morang district in Koshi Zone in Nepal on 15 May 2011. The plant was identified by Tilak Gautam, and a voucher specimen (1100) has been deposited in the herbarium of the Tribhuvan University, Post Graduate Campus, Botany Department, Biratnagar. The fresh sample of the aerial parts (100 g) was hydrodistilled to obtain the essential oil in 0.5% yield.

2.2 Gas Chromatography – Mass Spectrometry

The *A. conyzoides* essential oil was analyzed by GC-MS using an Agilent 6890 GC, Agilent 5973 mass detector, and HP-5ms column as described previously [16]. Identification of the essential oil components was based on their retention indices, determined with reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentations with those in the literature [17] and our own in-house reference library.

2.3 Hierarchical Cluster Analysis

The essential oil compositions of 22 *A. conyzoides* samples, including this work from Nepal and 21 compositions reported in the literature [18–34], were used to carry out a cluster analysis using XLSTAT (v. 2017.5.47159). The essential oil compositions were treated as operational taxonomic units (OTUs) and the percentages of 20 of the most abundant components (precocene I, precocene II, β-caryophyllene, (*Z*)-β-farnesene, caryophyllene oxide, germacrene D, (*E*,*E*)-α-farnesene, β-sesquiphellandrene, coumarin, α-humulene, cubebene, bicyclogermacrene, 6-vinyl-7-methoxy-2,2-dimethylchromene, γ-cadinene, 6-(1′-hydroxyethyl)-2,2-dimethylchromene, β-bourbonene, 6-(1′-hydroxyethyl)-7-methoxy-2,2-dimethylchromene, bornyl acetate, α-pinene, and δ-cadinene) were used to establish the chemical relationships of the essential oils of *A. conyzoides* using the agglomerative hierarchical cluster (AHC) method. Pearson correlation was used to measure similarity and the unweighted pair-group method with arithmetic average (UPGMA) was used to define the clusters.

2.4 Bioactivity Screening

The *A. conyzoides* essential oil was screened for antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and for antifungal activity against *Aspergillus niger* as previously described [16]. The essential oil of *A. conyzoides* was screened for nematicidal activity using *Caenorhabditis elegans* and for brine shrimp (*Artemia salina*) lethality as previously reported [35].

3. Results and Discussion

The essential oil from the aerial parts of *A. conyzoides*, collected from Biratnagar, Nepal, was obtained in 0.5% yield as a clear yellow oil. Gas chromatographic – mass spectral analysis of the oil revealed 40 identifiable components representing 99.7% of the oil composition (Table 1). Precocene I (6-demethoxyageratochromene, 61.7%) and precocene II (ageratochromene, 23.5%) dominated the essential oil, with lesser concentrations of 6-(1′-hydroxyethyl)-2,2-dimethylchromene) (6.0%), and 6-(1′-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (4.3%). Monoterpenoids made up a negligible percentage of the composition.

Table 1: Essential oil composition of *Ageratum conyzoides* from Nepal.

RI	Compound	%
854	(2*E*)-Hexenal	tr
856	(3*Z*)-Hexenol	tr
867	(2*Z*)-Hexenol	tr
869	1-Hexanol	tr
941	α-Pinene	tr
953	Camphene	tr
963	Benzaldehyde	tr
976	Sabinene	tr
978	β-Pinene	tr
981	1-Octen-3-ol	tr
1024	*p*-Cymene	tr
1028	Limonene	tr
1030	1,8-Cineole	tr
1032	Benzyl alcohol	tr
1041	Salicylaldehyde	tr
1043	Phenylacetaldehyde	tr
1100	Linalool	tr
1112	2-Phenylethyl alcohol	tr
1124	Chrysanthenone	0.1
1164	Borneol	tr
1176	Terpinen-4-ol	tr
1189	α-Terpineol	tr
1193	Methyl salicylate	tr
1225	Bornyl formate	tr
1285	Bornyl acetate	0.1
1311	*p*-Vinylguaiacol	0.2
1357	Eugenol	0.3

1419	β-Caryophyllene	0.1
1437	Coumarin	2.0
1464	Precocene I (= 6-Demethoxyageratochromene)	61.7
1565	(*E*)-Nerolidol	0.1
1586	*trans*-Sesquisabinene hydrate	0.1
1590	Caryophyllene oxide	0.2
1622	6-(1′-Hydroxyethyl)-2,2-dimethylchromene	6.0
1637	Caryophylla-4(12),8(13)-dien-5α-ol	0.1
1641	Caryophylla-4(12),8(13)-dien-5β-ol	0.2
1660	Precocene II (= Ageratochromene)	23.5
1677	6-Vinyl-7-methoxy-2,2-dimethylchromene	0.5
1693	Tridecyl acetate	0.2
1823	6-(1′-Hydroxyethyl)-7-methoxy-2,2-dimethylchromene	4.3

In order to place the chemical composition of the *A. conyzoides* of Nepalese origin into perspective with essential oils from other geographical locations, a hierarchical cluster analysis, based on the chemical compositions of *A. conyzoides* previously reported in the literature was carried out. The cluster analysis revealed two distinct clusters, (I) dominated by precocene I, and (II) having a more equitable distribution of precocene I and precocene II (Fig. 1). Interestingly, there seems to be little correlation between geographical origin or plant tissue (flowers, leaves, aerial parts) with chemical composition.

Ageratum conyzoides essential oil from Nepal was screened for antibacterial activity, antifungal activity, nematicidal activity, and brine shrimp lethality (Table 2). The essential oil showed only marginal (*B. cereus*, *A. niger*) to no activity in the assays. Patil and co-workers found *A. conyzoides* essential oil from Kolhapur, India, to be weakly antifungal against *A. niger* with MIC = 1500 μg/mL [28]. These workers also carried out antibacterial screening using the disk diffusion assay, but MIC values were not determined. Likewise, Adjou and co-workers reported MIC values of 2000 μg/mL and 2500 μg/mL for *A. conyzoides* essential oil from Benin against *Aspergillus flavus* and *A. parasiticus*, respectively [19]. Liu and Liu carried out larvicidal activity of *A. conyzoides* essential oil from Fuzhou City, China, on *Aedes albopictus* larvae and determined the LC_{50} to be 61.2 μg/mL [23].

Table 2: Biological activity screening of *Ageratum conyzoides* essential oil from Nepal.

Antimicrobial (MIC, μg/mL)		Lethality assays (LC_{50}, μg/mL)	
Bacillus cereus	313	*Caenorhabditis elegans*	212
Staphylococcus aureus	2500	*Artemia salina*	> 100
Escherichia coli	2500		
Pseudomonas aeruginosa	625		
Aspergillus niger	313		

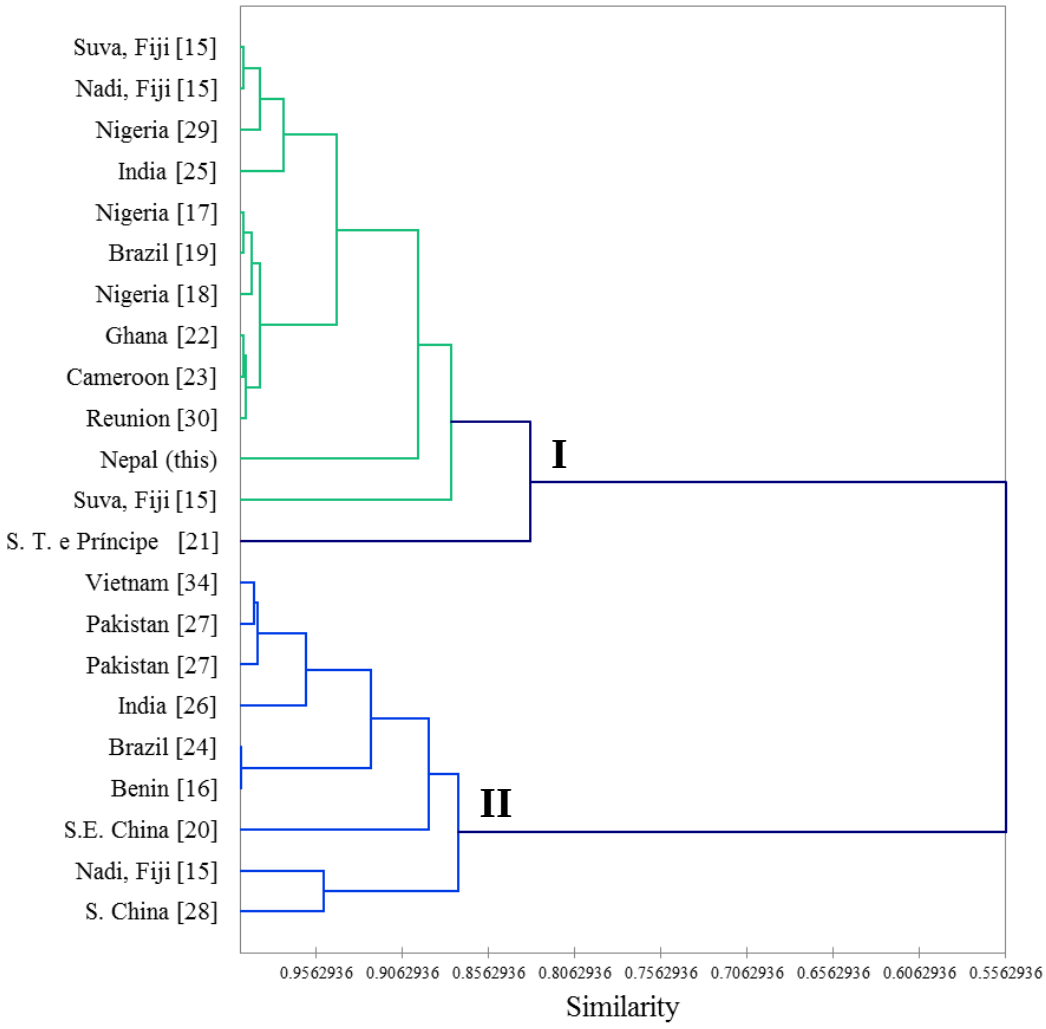

Figure 1: Dendrogram obtained from the agglomerative hierarchical cluster analysis of 22 *Ageratum conyzoides* essential oil compositions.

4. Conclusions

The essential oil from the aerial parts of *Ageratum conyzoides* from Biratnagar, Nepal was dominated by precocene I. There are two apparent clusters based on the essential oil compositions of *A. conyzoides*, (I) dominated by precocene I, and (II) having a more equitable distribution of precocene I and precocene II, and the sample from Nepal falls into cluster (I). The essential oils of *A. conyzoides* have shown only marginal biological activities.

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