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RESEARCH ARTICLE

Chemical Constituents of the Essential Oil of Invasive *Chromolaena odorata* leaves in Central Nepal

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Abstract

Chromolaena odorata is one of the widely distributed invasive alien plants in the tropical to subtropical regions of Nepal. It has the ability to impact native species in its invaded range by multiple modes such as allelopathy including volatilization. This study aims to identify volatile chemical components in the essential oil of *C. odorata*. The leaf samples of *C. odorata* were collected from the Radha Krishna Community Forest, Chitwan district of Nepal. The essential oil from the leaves was extracted and the chemical composition of the oil was analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). A total of 19 chemical components from the oil were identified. The major components were linalool (21.64%); β-pinene (9.43%); 1,3-cycloheptadiene (8.92%); β-cubebene (7%); cinnamaldehyde (5.30%) and caryophyllene oxide (4.94%). To the best of our knowledge, the presence of o-methoxy cinnamaldehyde and isoeugenyl acetate was not listed as the chemical constituents in volatile oils of *C. odorata* previously. The components of the volatile oil are suspected to have an allelopathic effect on native species, anti-herbivory properties, and medicinal values. Therefore, this study could be important to understand plant invasiveness and utilization of the plant for the extraction of bioactive compounds that could contribute to control and manage the invasive plants in the invaded areas.

Keywords: Invasive plant, Siam weed, essential oil, GC-MS analysis, phytotoxicity, allelopathy

Introduction

Chromolaena odorata (L.). R. M. King and H. Rob. (syn. *Eupatorium odoratum* L.), Siam weed, is a highly problematic invasive alien plant species in Nepal. It is native to tropical America and Mexico, and currently, it has been naturalized in many countries of the world including Nepal [1]. It is one of the rapidly growing perennial shrubs of the family Asteraceae. This species is one among 100 of the world's worst invasive alien species [2]. It is distributed from tropical to sub-tropical regions (75m-1700m) of eastern to western Nepal [3]. Massive colonization of *C. odorata* is found in forest margins, fallow lands, roadsides, and even in the agro-ecosystems [3-6]. As one of the troublesome, aggressive, and noxious weeds, it has threatened native biodiversity, impacted peoples' livelihood and economy [3-5].

Chromolaena odorata produces many allelochemicals such as terpenoids, phenolics, and alkaloids from its underground and aerial parts [7,8]. The allelochemicals produced by this plant are known to be toxic for seed germination, growth, and development of native plant species [9]. The allelochemicals are also toxic to livestock [10]. The chemical constituents of its volatile oil such as terpenoids are known as the harmful allelochemicals [11]. These chemicals are one of the mechanisms of harming native species, as a result, the invasive species grow faster in their novel range [12]. Medicinally, the plant oil has the potential to remedy diseases such as treatment of malaria, wounds, stomach disorders, skin diseases, coughs, and colds, etc. [13]. Besides, the essential oil of this species is insecticidal [14], used as an insect repellent [15], and antibacterial [16,17]. Therefore, the volatile components of this species have an important role in plant invasiveness, toxicity, and medicine.

A survey of the literature revealed that the essential oil of *C. odorata* contains several volatile chemicals and other biologically active constituents [18-21]. Documentation of chemical constituents of *C. odorata* volatile oil has been carried out from different countries such as Nigeria [19], Thailand [20], Malaysia [21], Vietnam [22], Ivory Coast [23], Benin [24], and Ghana [25], etc. Such documentation from Nepal is very meager. It is an urgent task to know the chemical constituents of such invasive plants in Nepal to understand whether the constituents vary with geographical regions. Hence, this study aims to document the chemical components in the volatile oil of *C. odorata* leaves from the Tarai region of central Nepal.

Materials and Methods

Collection of plant materials

Fresh leaves of *C. odorata* were collected from the invaded site of the Radha Krishna Community Forest (27° 34' 59.99"N and 84° 30' 59.99"E, 127 masl) located at Meghauli-26, south-western part of Bharatpur Metropolitan City, Chitwan, Nepal in July 2019. The samples were stored in the refrigerator (4°C). The voucher specimen has been deposited at the Tribhuvan University Central Herbarium (TUCH), Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Extraction of essential oil and chemical analysis

The essential oil from the collected leaf sample was extracted by using Clevenger apparatus (300 ml) for about 3 hours at 80°C by the process of hydro-distillation at Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Figure 1: *Chromolaena odorata plant photograph*

The light yellow colored extracted oil was collected and transferred to a clean glass vial, sealed, and stored at a low temperature (0-4^{o}C) until further analyses.

The components of the essential oil were separated by Gas Chromatography (GC) and identified by Mass Spectrometry (MS). The GC-MS analysis was carried out at the Department of Food Technology and Quality Control, Government of Nepal, Babarmahal, Kathmandu, Nepal. The Shimadzu QP2010 Plus operating system in electron impact (EI) mode was used for GC-MS analysis. The essential oil was subjected to a gas chromatograph fitted with an Rtx-5MS column (30 m×0.25 mm i.d., film thickness 0.25 µm), coupled to a QP2010 Plus mass detector. The column oven temperature was kept at 80°C for 2.0 minutes then gradually raised to 280°C at a rate of 6°C/min and finally held isothermally for 5 minutes. The injector temperature was set at 220°C. Helium was used as a carrier gas at a total flow rate of 18.5 mL/min. One microliter of the diluted oil samples (1/100 in ethanol, *v*/*v*) were injected manually (split mode, split ratio 1:15) with 1.03 mL/min through the column. For GC-MS detection, an electron ionization system with ionization energy of 70 eV on an Rtx-5MS mass spectrometer connected to GC-MS solution software was used. Spectra were obtained over the mass range of 40 to 500 m/z at scan speed 1000 millisecond. The relative percentage composition of individual component was calculated from the GC peak area of all the components of the chromatograph.

Qualitative and quantitative analysis

The relative quantity of the chemical components present in the extracts of *C. odorata* was expressed as a percentage based on the peak area produced in the chromatogram. The identification of compounds was accompanied by comparing with the National Institute of Standards and Technology (NIST 05S) library. The quantification of the compounds was determined by the relative percentage area of the peaks.

Results and Discussion

A total of 19 components were identified from the oil of *C. odorata.* The list of the components, R time, area %, and base m/z ratio have been given in Table 1 and the total ion chromatogram (TIC) is given in Figure 2. Three components, linalool (21.64%), β-Pinene (9.43%), and 1, 3-cycloheptadiene (8.92%) were the major compounds having a high percentage comparing to other components in the oil. Other compounds β-cubebene, cinnamaldehyde, and caryophyllene oxide occupied about 4 to 7% (Table 1). Rest, caryophyllene, cinnamyl acetate, β-ocimene, D-limonene, o-methoxy cinnamaldehyde, bornyl acetate, δ-cadinene, isoeugenyl acetate, humulene, cadinol, terpinen-4-ol, and p-cymene were the compounds having low concentrations i.e. <4% (Table 1).

Table 1: *The list of components, R time, area %, and base m/z ratio of volatile oil of C. odorata*

Compounds	R Time	Area %	Base m/z
β-Pinene	4.356	9.43	93.10
p-Cymene	5.139	1.18	119.10
D-Limonene	5.213	2.57	68.05
β-Ocimene	5.518	3.04	93.10
Linalool	6.62	21.64	71.05
Terpinen-4-ol	8.327	1.37	71.10
Cinnamaldehyde	10.402	5.30	131.10
Bornyl acetate	10.7	1.77	95.10
1,3-Cycloheptadiene	10.855	8.92	79.05
Copaene	12.65	0.63	105.10
Caryophyllene	13.611	3.66	69.10
Cinnamyl acetate	14.074	3.22	43.05
Humulene	14.32	1.53	93.10
β-Cubebene	14.892	7.00	161.10
δ-Cadinene	15.69	1.75	161.10
o-Methoxycinnamaldehyde	15.865	2.50	131.10
Caryophyllene oxide	16.966	4.94	79.05
Cadinol	17.896	1.50	119.10
Isoeugenyl acetate	18.507	1.66	163.05

Comparing to the prior literature, the types and concentrations of chemical components in present study are distinct. Ling *et al.*, (2003) had found trans-caryophyllene (16.58%), δ-cadinene (15.85%), α-copaene (11.58%), caryophyllene oxide (9.63%), germacrene-D (4.96%), and δ-humulene (4.32%) as the major compounds in *C.odorata* essential oil [11]. Similarly, Owolabi et al. (2010) had identified α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (E)-caryophyllene (5.4%), and geijerene/pregeijerene (7.5%) as the major components [19]. The major components identified by Pisutthanan *et al.,* (2006) were pregeijerene (17.6%), germacrene D (11.1%), α-pinene (8.4%), β-caryophyllene (7.3%), vestitenone (6.5%), β-pinene (5.6%), δ-cadinene (4.9%), geijerene (3.1%), bulnesol (2.9%), and *trans*-ocimene (2.2%) [20].

The major components identified by Félicien et al.

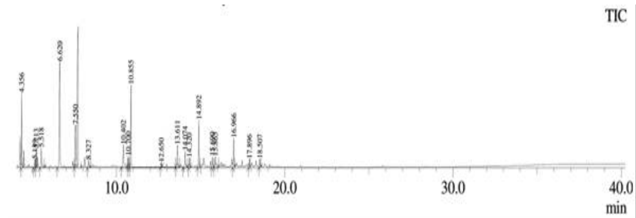

Figure 2: *Total ion chromatogram (TIC) of volatile oil of C. odorata*

(2012) were similar to the findings of Pisutthanan et al. (2006) but there was variation in concentrations of the components [20,26]. Contrasting to these literature, Joshi (2013) had reported himachalol (24.2%), 7-isopropyl-1,4-dimethyl-2-azulenol (17.6%), and 2-methoxy-6-(1-methoxy-2-propenyl) naphthalene (5.6%) as the major compounds from *C. odorata* in India [18]. The major components identified in our study were linalool (21.64%), β-pinene (9.43%), and 1, 3-cycloheptadiene (8.92%) which were quite different than the findings of others. Interestingly, the present study indicates that the types and amount of major components in the volatile oil vary with geographical locations.

The compounds such as β-pinene, copaene, δ-cadinene, caryophyllene, caryophyllene oxide, humulene, and ocimene identified from our study were common with the findings of previous studies [19,20,24-26] but the presence of o-methoxy cinnamaldehyde and isoeugenyl acetate were not listed as the chemical constituents in essential oils of *C. odorata* in aforementioned studies. The mass spectra and chemical structure of o-methoxy cinnamaldehyde and isoeugenyl acetate are given in Figure 3. Thus, these two compounds should be further confirmed as the new report from the C. odorata collected from Tarai region of central Nepal.

As *C. odorata* has negative effects on native plant species, forage for livestock, crops, soil and soil microbes, it is one of the worst invasive plants [4,27,28]. And, allelopathy is one of the important mechanisms of the plant to affect negatively other species [31]. Previous studies have confirmed that the allelochemicals present in aerial and underground parts of *C. odorata* are phytotoxic to other plants [7,29]. Certain allelochemicals (components of volatile oils) such as cadinene, caryophyllene, pinene, limonene, etc. are found to be phytotoxic compounds [30-32]. Therefore, the presence of these compounds in this invasive weed might have harmed the Nepalese native plants in its invaded range.

On the other hand, the compounds such as β-pinene, β-cubebene, β-ocimene, and linalool are the volatile terpenes released in response to herbivory by several

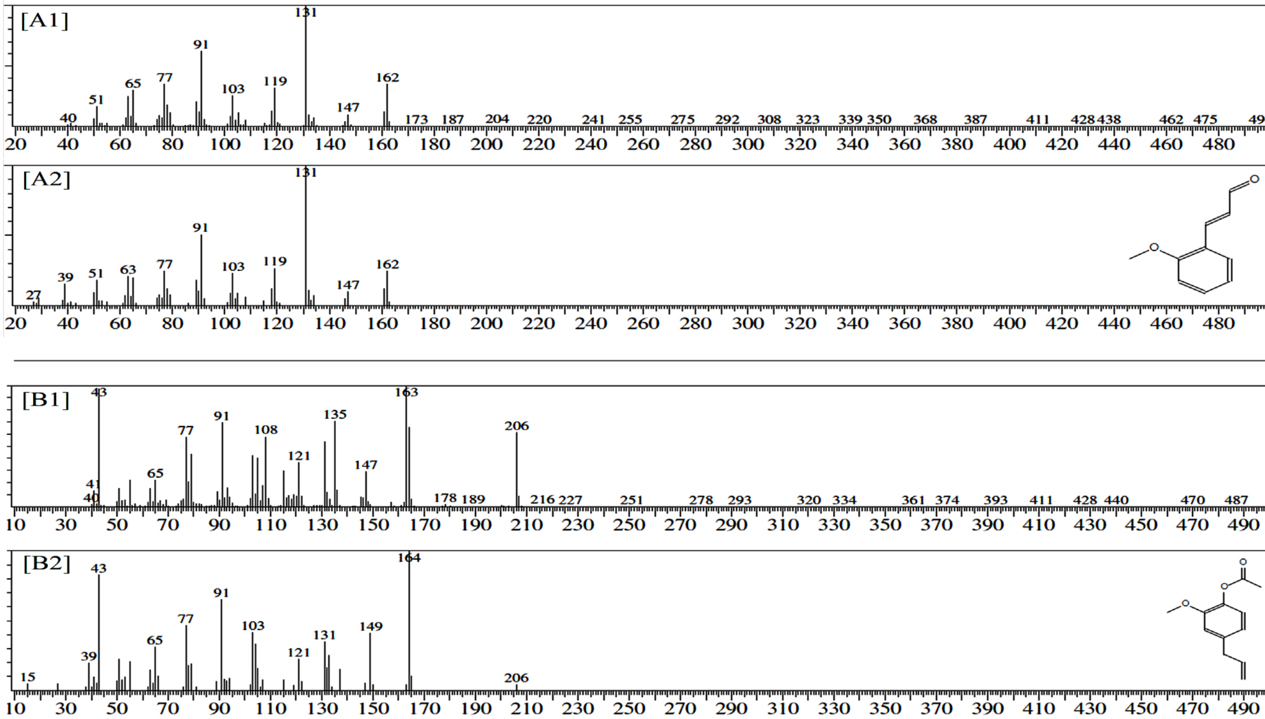

Figure 3 *Mass spectra of o-methoxy cinnamaldehyde [A1] and isoeugenyl acetate [B1] obtained from the volatile oil of C. odorata and the spectra of same compounds obtained from NIST library [A2 and B2]*

plants [33-35]. As these compounds are found in the leaves of *C. odorata,* it is expected that these defensive chemicals are used by this plant against herbivory damage. Meanwhile, less damage by herbivores to invasive plants is considered as one of the mechanisms for successful growth in a novel range [36,37]. Hence, the abundance of anti-herbivory chemicals in *C. odorata* is one of the causes of successful growth in the native environment. Therefore, exploration of such allelochemicals would have great significance to understand the plant invasiveness as well as their potential impacts on the native environment.

Moreover, control and management of the naturalized *C. odorata* have become a challenging issue throughout the world. It is often suggested that the utilization of such invasive plants can be one way to manage and control them sustainably. Several studies show that *C. odorata* is used as a medicinal plant as it has many ethnopharmacological uses, for example, treatment of malaria, wounds, stomach disorders, skin diseases, coughs, and colds, etc. [13]. There might be a great role of several compounds present in *C. odorata* for the treatments of such diseases. As an example, linalool is anti-inflammatory, anticancer, anti-hyperlipidemic, antimicrobial, analgesic, anxiolytic, antidepressive, and neuroprotective properties [38]. Hence, *C. odorata* can be utilized for the extraction of such high valuable medicinal compounds.

Conclusion

The present study identified 19 chemical compounds from the essential oil of *C. odorata* leaves collected from the Radha Krishna Community Forest of Meghauli, Chitwan, Nepal. The major compounds were linalool; β-pinene; 1,3-cycloheptadiene; β-cubebene; cinnamaldehyde, and caryophyllene oxide. The study indicates that the types and amount of major components in the volatile oil vary with geographical locations. Importantly, the presence of o-methoxy cinnamaldehyde and isoeugenyl acetate in this study may be the new report from the *C. odorata*. Most of the components of the volatile oil identified are allelochemicals, anti-herbivory, and medicinal. Utilization of *C. odorata* for the extraction of bioactive compounds can contribute to the control and management of this plant in Nepal.

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