VOLATILE COMPOUNDS AND BIOLOGICAL ACTIVITY OF *Chromoleana odorata* AND *Ageratum conyzoides* ESSENTIAL OILS

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ABSTRACT

Chromoleana odorata (L.) R. M. King and *Ageratum conyzoides* L. (Asteraceae) are medicinal-aromatic plants growing in the wild. The leaves volatiles were isolated and characterized by means of hydro-distillation and gas chromatography-mass spectrometry (GC-MS) respectively. *C. odorata* oil comprised mainly of pregeijerene (15.8%), β-caryophyllene (11.1%), germacrene D (20.0%), and δ-cadinene (10.6%). Precocene 1 (79.8%) and β-Caryophyllene (10.6%) occurred as major compounds of *A. conyzoides* leaves. The leaf oils demonstrated moderate free radical scavenging activity compared to ascorbic acid using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical model.

INTRODUCTION

Essential oils (the main class of phyto-constituents of aromatic plants), are hydrophobic liquids containing volatile fragrant aroma compounds (Wanda, 2001). Essential oils from medicinal plants have been exploited as antioxidant, antimicrobial, anticancer, antidiarrheal, antihypertensive, antidiabetic, anti-inflammatory, immunomodulatory, and insecticidal agents (Hamid *et al*., 2011). *Chromolaena odorata* (L.) R. M. King (Syn: *Eupatorium odoratum* L.) and *Ageratum conyzoides* L. are invasive aromatic perennial shrubs belonging to the plant family Asteraceae. Medicinally, the plants decoction is taken as a remedy for cough and cold or in baths to treat skin diseases. They are used as wound healing and local antiseptic agents (Inya-Agha *et al*., 1987; Iwu, 1993). A number of researchers have reported the composition of *C. odorata* from different regions of the world (Inya-Agha *et al*., 1987; Lamaty *et al*., 1992; Bamba *et al*., 1993; Sohounhoue *et al*., 1996; Bedi *et al.*, 2001; Chowdhury, 2002; Pisutthanan *et al*., 2006). Likewise, analysis of the *A. conyzoides* essential oils from different countries showed significant variations in the oil constituents (Kong *et al*., 1999; Sundufu and Huang, 2004; Lima *et al*., 2005; Martins *et al*., 2005). For instance, the oil obtained from *A. conyzoides* in India was of ageratochromene chemotype (Padalia *et al*., 2010), while that of Reunion was characterized by the abundance of demethoxyageratochromene and β-caryophyllene respectively (Vera, 1993). The essential oils of *C. odorata* and *A. conyzoides* have been poorly studied in Nigeria with respect to composition and pharmacological applications (Owolabi *et al*., 2010). In this report, we present the composition and antioxidant activity of *C. odorata* and *A. conyzoides* leaves essential oils.

MATERIALS AND METHODS

Plant Sample and Isolation of Essential Oils

Chromolaena odorata and A. conyzoides were collected within the vicinity of University of Uyo, Nigeria. The plants were identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The fresh leaves were cut in pieces, pulverized, and essential oils were obtained by hydro-distillation (4 h) using a Clevenger-type apparatus in accordance with the British Pharmacopoeia (Anonymous, 1980). The oils were dried over sodium sulfate (Sigma-Aldrich, St. Louis, MO, USA) and stored in a refrigerator (4 °C) after the estimation of percentage yield.

Gas Chromatography-Mass Spectrometry Analyses (GC-MS)

GC-MS analyses were performed with a Varian CP- 3800 gas chromatograph, equipped with a HP-5 capillary column (30 m ×0.25 mm; coating thickness, 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 ml/min; injection of 0.2 μL (10 % hexane solution); split ratio of 1:30. Constituents' identification was based on comparison of retention times with those of authentic samples (Sigma-Aldrich, USA); this implied comparing their linear retention indices (LRIs) with the series of *n*-hydrocarbons and using computer matching against commercial and home-made library mass spectra (built up from pure substances and components of known oils and mass spectra literature data (Adams, 1995).

2,2-Diphenyl-1-Picrylhydrazyl(DPPH) Radical Scavenging Activity

The DPPH free radical scavenging of the essential oils and ascorbic acid prepared in methanol at various concentrations (20–100 μg/mL) were evaluated according to the method of Shekhar and Anju (2014). One millilitre of 0.1 mM DPPH solution in methanol was added to 3 mL of solutions prepared with the extract and standard, and stirred for 1 min. Each mixture was kept in the dark at room temperature for 30 minutes and the absorbance was recorded against a blank at 517 nm. The assays were carried out in triplicate and the results were expressed as mean values ± standard error of mean. Lower absorbance of the reaction mixture indicated higher free radical activity. Percentage scavenging activity was calculated using the expression:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

The volatile compounds of *C. odorata* and *A. conyzoides* leaves are presented in Table 1. Oil yields of 0.9% and 0.8% w/v were recorded for leaves of *C. odorata* and *A. conyzoides* respectively.

Forty constituents were identified in the leaf oil of *C. odorata*, accounting for 98.1% of total oil composition; while the twenty components in the leaf oil of *A. conyzoides* constituted 99.6% of the total oil content. *C. odorata* leaf oil comprised mainly of germacrene D (20.0%), pregeijerene (15.8%), β-caryophyllene (11.1%), and δ-cadinene (10.6%); while precocene 1 (79.8%) and β-caryophyllene (10.6%) occurred as major compounds of *A. conyzoides* leaves. The percent composition of various classes of compounds in *C. odorata* and *A. conyzoides* respectively were: monoterpenoid (10.8 and 0.0%), sesquiterpenoid (87.3 and 99.2%), oxygenated terpenes (9.0 and 1.1%), and non terpene derivatives (0.0 and 0.4%). Though variations in oil composition of *C. odorata* and *A. conyzoides* from some regions of the world are documented (Vera, 1993; Padalia *et al*., 2010; Felicien *et al*., 2012; Pitakpawasutthi *et al*. 2016), generally, reports indicate high monoterpene and sesquiterpene content for essential oils of *C. odorata*. Likewise, the composition of *A. conyzoides* oils are reported low in oxygenated compounds.

The DPPH radical scavenging activities of *C. odorata* and *A. conyzoides* oils, compared with the standard compound (ascorbic acid), is presented in Figure 1. The plot in Figure 1 indicates the scavenging ability as percent inhibition at various concentrations (20–100 μg/mL); the scavenging effect was concentration dependent. *C. odorata* and *A. conyzoides* oils demonstrated moderate activity (46.8% and 48.3% free radical inhibition at 100 μg/mL, respectively), compared with ascorbic acid (90.5%). A report has suggested a relationship between the free radical scavenging activity of polyphenolic substances and their chemical structures (Samak *et al*. 2009), hence the ability of constituents inherent in the extract to act as hydrogen atoms or electrons donor in the conversion of the stable purple coloured DPPH to the reduced yellow coloured DPPH-H.

Table 1: Volatile constituents of *C. odorata* and *A. conyzoides* leaves

Constituent	LRI[a]	Relative Abundance (%)[b,c]		Constituent	LRI[a]	Relative Abundance (%)[b,c]	
		C. O	A. C			C. O	A. C
α-Pinene	941	4.7	-	Bicyclosesquiphellandrene	1498	0.8	0.4
Sabinene	977	0.5	-	Bicyclogermacrene	1496	3.6	0.9
β-Pinene	982	3.0	-	α-Muurolene	1499	1.1	-
Myrcene	993	0.7	-	Germacrene A	1505	1.0	0.1
Limonene	1032	0.5	-	δ-Amorphene	1506	0.3	-
(*Z*)- β-Ocimene	1042	0.2	-	*(E,E)*-α-Farnesene	1508	0.2	-
(*E*)-β-Ocimene	1052	1.2	-	*trans*-γ-Cadinene	1514	0.6	-
Geijerene	1142	2.7	-	Cubebol	1515	0.4	-
Pregeijerene	1288	15.8	-	δ-Cadinene	1524	10.6	-
α-Copaene	1377	3.2	0.1	β-Sesquiphellandrene	1525	-	2.3
β-Bourbonene	1385	0.2	0.2	Elemol	1550	0.6	-
β-Cubebene	1391	-	0.4	Germacrene B	1557	0.6	-
β-Elemene	1392	0.8	0.2	*(E)*-Nerolidol	1564	0.2	0.2
β-Caryophyllene	1419	11.1	10.6	Caryophyllene oxide	1582	1.0	0.5
β-Copaene	1430	0.4	-	Viridiflorol	1591	0.7	-
trans-α-Bergamotene	1437	-	0.2	Humulene epoxide II	1607	0.2	-
Aromadendrene	1440	-	0.3	Humulane-1,6-dien-3-ol	1616	0.2	-
cis-Muurola-3,5-diene	1448	0.2	-	1-*epi*-Cubenol	1629	0.7	-
α-Humulene	1455	3.4	0.8	γ-Eudesmol	1632	0.5	-
(E)-β-Farnesene	1460	-	0.2	T-Cadinol	1641	1.7	0.2
Precocene I	1466	-	79.8	β-Eudesmol	1651	0.4	-
trans-Cadina-1(6),4-diene	1475	0.7	-	α-Cadinol	1653	2.4	0.2
γ-Muurolene	1478	1.0	-	Androencecalinol	1676	-	0.4
Germacrene D	1482	20.0	1.6	Total identified		98.1	99.6

[a]LRI, Linear retention indices; [b]Order of elution on HP-5ms capillary column; [c]Identification by comparison of the mass spectral and retention index data; C.O, *C. odorata*; A.C, *A. conyzoides*; - = not detected

The low antiradical effect of the essential oils may be attributed to their chemical composition (9.0 and 1.1% oxygenated compounds). Felicien *et al*. (2012) and Pitakpawasutthi *et al*. (2016) reported low DPPH radical scavenging for *C. odorata* essential oils; the leaf oil composed mainly of monoterpenes and sesquiterpenes. Similarly, Bayala *et al*. (2014) indicated low percent inhibition of DPPH free radicals for leaf essential oil of *A. conyzoides* from Bukina Faso.

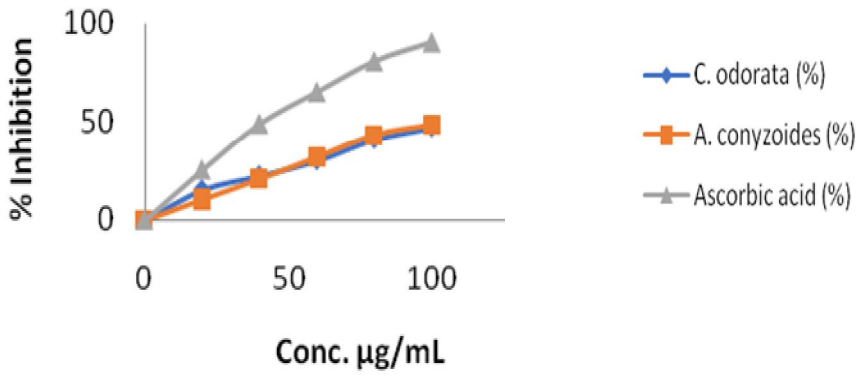

Figure 1: DPPH radical scavenging activity of *C. odorata* and *A. conyzoides* oils

CONCLUSION

The chemical composition of the essential oils of *C. odorata* and *A. conyzoides* are reported. *C. odorata* and *A. conyzoides* volatile oils exhibited moderate antioxidant activity which may be attributed to the low concentration of oxygenated compounds.

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