Physical and Chemical properties, Composition, and Biological Activity of Essential Oils of Philippine Medicinal Plants

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ABSTRACT

The composition, sensory and physical/chemical properties of essential oil from *Chromolaena odorata* (L.) R.M. King & H. Rob., *Mikania micrantha* Kunth, and *Ipomoea pes-caprae* (L.) R. Br. of Philippine origin were analyzed by GC/MS. and the oil was evaluated using brine shrimp lethality assay (BSLA). The GC/MS analysis revealed a composition of 58 sesquiterpenes that represented more than 90 percent of the total oil extracted from the dry leaves of *C. odorata*. The major components were caryophyllene oxide (11.41%), β-caryophyllene (10.9%), δ-cadinene (10.59%), germacrene D (9.87%), α-copaene (3.01%), cadinenol (2.30%), α-trans bergamotene (1.99%), and α-humulene (3.18%).

Fifty six compounds were detected from the essential oil in the dried leaves of *M. micrantha*. The main components of essential oil from the plant contained sesquiterpenoids specifically, β-cubenene (6.31%), δ-cadinene (6.16%), caryophyllene oxide (6.71%), e-nuciferol (4.45%), α-muurol (4.13%), α-bisabolol (4.05%), spathulenol (2.98%) (2.98%), β-bisabolene (2.59%), and E-caryophyllene (2.19%).

A total of 32 compounds were detected in the essential oil identified from the dried leaves of *I. pes-caprae*. The major compound identified was β-caryophyllene that comprised 46.30% of the total relative volatile oil percent composition. The other major constituents identified were a-humulene (7.31%), δ-cadinene (6.16%), germacrene D (7.63%) and caryophyllene oxide (9.11%). The *C. odorata, M. micrantha,* and *I. pes-caprae* essential oil showed cytotoxic activity when checked with the brine shrimp lethality assay with LD50 of 52.16 µg/mL, 101.38 µg/mL and 92.50 µg/mL, respectively. These essential oils could be used as ingredients for the development of new natural plant products

INTRODUCTION

Since ancient times, essential oils extracted from plants have been used in many cultures due to their medicinal value and health benefits. Currently, these oils have become basic components in numerous industrial products, including perfumes, food and beverage flavorings, and a wide range of biopharmaceuticals (Djilanil and Dicko, 2014). Recent trends in health care have focused on holistic health practices and are geared towards rediscovering the beneficial effects of essential oils

Essential oils have multiple applications in medicine, pharmaceuticals, cosmetics, and industries characterized by increasing consumption and production (Yentema, et al., 2007). The unique properties of essential oils include cleansing properties and a variety of biological properties. Some essential oils have potent health benefits and are used as dietary supplements to promote vitality and wellness. Thus, production technology is critical to produce quality essential oils (Hill, 2011).

The Philippines are one of the tropical countries in the world endowed with an abundance of plant biodiversity. *Chromolaena odorata* (L.) R.M. King & H. Rob., *Mikania micrantha* Kunth, and *Ipomoea pes-caprae* (L.) R. Br. are used in Philippine folkloric medicine. About 450 species of *Mikania* (Asteraceae), 165 species of *Chromolaena* (Asteraceae), and over 500 species of *Ipomaea* (Convolvulaceae) have been identified (Olusegun and Musa, 2014). The plants are fast growing and are wide spread in Philippines and Asia.

In native medicine, the plants are used in the treatment of coughs, colds, and skin diseases (Blanco, 1996; Morton, 1981).

Chromolaena odorata is a herbal plant with diverse pharmacological activities including anthelmintic, antimalarial, analgesic, anti-inflammatory, antipyretic, antispasmodic, antigonorrheal, antimycobacterial, insecticidal, fungicidal, wound healing, diuretic, blood coagulation, antidiabetic, and antibacterial activities (Chakraborty, et al., 2011; Panda, et al., 2012; Phn, et al., 2001).

Mikania micrantha exhibits antitumor, cytotoxic, analgesic, anti-inflammatory, antiproliferative, and phytotoxic activities (Bakir, et al., 2004; Facey, et al., 1999; Zhuang, et al., 2010). *Ipomea pes-caprae* (Convolvulaceae) is used to treat rheumatism and stomachic problems, as tonic, astringent, diuretic, laxative, antispasmodic, antihistaminic, hypoglycemic, inhibition of platelet aggregation, diarrhea, and hemorrhoids (Kumar, et al., 2014).

Research studies have shown that the chemical compositions of the essential oils of *C. odorata* of Cameroon and Congo mainly contained α-pinene and *p*-cymene (Lamaty, et al., 1992), while in India, the plants contain pregeijerene, epi-cubebol, cubebol, cis-sabinene hydrate, 10-epi-γ-eudesmol, germacrene-D-4-ol, α-cadinene, germacrene D, geijerene, cyperene, 10-epi-α-eudesmol, α-muurolol and khusimone (Joshi, 2013). The major compounds, such as α-pinene, geijerene, and pregeijerene from Ivory Coast have been identified (Bedi, et al., 2001; 2004). *Chromolaena odorata* of Thailand origin, contains pregeijerene, germacrene D, α-pinene, β-caryophyllene, vestitenone, β-pinene, α-cadinene, geijerene, bulnesol, and trans-ocimene as the major constituents (Shimizu and Tomoo, 1994).

The major components of *I. pes-caprae* in Mauritius were 8-cedren-13-ol (13.0%), (E)-nerolidol (7.0%), guaiol (6.2%), α-cadinol (6.2%) and limonene (6.1%) in fresh leaves and β-caryophyllene (36.6%), α-copaene (8.0%), germacrene D (7.3%), phytol (5.8%), δ-cadinene (5.7%), and α-humulene (5.4%) in the dried leaf samples (Marie, et al., 2007). The volatile oil from the seeds and inflorescence of *Mikania micrantha* from Mexico contain linalool and α-pinene as the main components (Pérez-Amador, et al., 2010).

No reports on the essential oil components and biological activity studies of *C. odorata. M. micrantha*, and *I. pes-caprae* in the Philippines are apparent. Thus, this research sought to determine the physical and chemical properties of essential oils from these groups of plants and to study their biological activities.

MATERIALS AND METHODS

Plant material. *Chromolaena odorata*, *Mikania micrantha*, and *Ipomea pes-caprae* leaves used in this study were collected within the localities of Cagayan de Oro City, Philippines., and authenticated by the Center of Biodiversity Research and Extension in Mindanao (CEBREM), Central Mindanao University (CMU), Musuan, Bukidnon, Philippines. Air-dried samples of the leaves were subsequently sent to the Department of Plant Biology and Pathology Laboratory, Rutgers, the State University of New Jersey, New Brunswick, NJ for study under a permit obtained from USDA.

Essential Oil Extraction. The essential oil from each set of the collected leaves was extracted by hydro-distillation using a Clevenger type apparatus. Briefly, 100 g of the powdered leaf tissue was added to a 2 liter round bottom flask to which 1 liter of deionized water was subsequently added. The flask was placed on a heating mantel for 2 h to extract the essential oil which was condensed and collected.

The collected essential oil from each plant was dried over anhydrous Na_2SO_4, transferred into sealed vials and refrigerated at <18°C until subsequent analysis. The yield of each essential oil was recorded as a percent of leaves dry weight and the quality of each essential oil was judged by the oil color.

Chemical composition. Each of the extracted essential oils was analyzed using a gas chromatograph (GC) coupled with a mass spectrometer (MS) and FID detectors (Juliani, et al, 2004). The identification of individual essential oil components was determined by matching MS component spectra to that in mass spectral libraries and validated by comparison with published relative indexes of the oil constituents (Adams, 2009).

Toxicity test. The toxic activity of essential oils was tested using a brine shrimp (*Artemia salina*) lethality assay (BSLA) adopted from Owokotomo, et al (2012) and Krishnaraju, et al (2005) with slight modifications. A seawater solution was prepared using a BSLA kit (Carolina Biological Supply Co.) by dissolving 10 g of sea salt in 2 L of distilled water at a pH 8.0. The seawater solution was subsequently placed in a plastic hatching chamber with a division for dark and light areas (fluorescent tubes providing light in the visible range, 400-600nm) (Van der Linden et al., 1987; Lavens and Sorgeloos, 1996). Shrimp eggs were placed in the hatching chamber and allowed to hatch for 48 h.

Sample test mixtures were prepared using stock solutions [a mixture of 20 mg of an essential oil with 0.3 mL of dimethylsulphoxide (1% DMSO)] brought to volume with 1.7 mL of fresh seawater solution to a 1000 µL/L essential oil constituent concentration. A dilution series of 1000, 100, 10, and 1 µL of essential oil were prepared for subsequent analysis of oil toxicity.

Brine shrimp in 3.0 mL of seawater-essential oil solution were transferred into the specimen test tubes prepared in triplicates. Then, 0.5 mL of each prepared concentration was transferred into the specimen test tubes followed by introduction of ten nauplii. As a control, ten nauplii were placed in specimen test tubes that did not contain essential oil. Following the transfer of the nauplii into the test tubes, the solution in each test tube was topped-up with seawater until the volume reached 5.0 mL. All tops of test tubes containing shrimp were left open for 24 h and exposed to light at 27 to 30 °C, after which time the number of surviving nauplii were counted and recorded with the aid of a dissecting microscope. The percentage mortality was calculated by comparing the mean nauplii of the nauplii of the test and control tubes. The LD_{50} values were calculated from the best-fit line plotted between the concentrations against percent lethality.

RESULTS

The chemical, physiochemical, and sensory properties of the essential oils extracted from the *C. odorata, M. micrantha,* and *I. pes-caprae* varied (Table 1). All three species were characterized by relatively low amounts of oil. *C. odorata* produced the highest oil yield (0.5%), followed by *M. micrantha,* and *I. pes-caprae* (0.4 and 0.3%, respectively). In terms of color, *C. odorata* produced blue tinted green colored oil, while *M. micrantha* oil was a light yellow color and *I. pes-caprae* was a bright yellow color. All three oils had densities lower than water , with *M. micrantha* having the highest density (0.8861). The species also showed the highest refractive index (1.4625), while *C. odorata* and *I. pes-caprae* showed lower values (1.4532 and 1.4412, respectively) (Table 1).

Table 1. Yields and physiochemical properties of Philippines vines.

Species	*C. odorata*	M. micrantha	*I. pes-caprae*
[1]Percent yield	0.5±0.18[2]	0.30±0.12	0.40±0.21
Appearance	Blue green	Light yellow	Bright yellow
Refractive index	1.4532	1.4625	1.4412
Density	0.8639	0.8861	0.8544

[1]Percent yield (mL/100 g dried tissue).

A total of 58 constituents were detected in the essential oils for *C. odorata* with the major essential oil components being sesquiterpenoids, including caryophyl-lene oxide (11.41%), β-caryophyllene (10.9%), δ-cadinene (10.59%), germacrene D (9.87%), α-copaene (3.01%) cadinenol (2.30%), α-trans bergamotene (1.99%) and α-humulene (3.18%) (Table 2). Other constituents in oil of *C. odorata* were at levels less than 3 percent of the total oil.

Major oil components in *M. micrantha* were an unknown at 6.71 percent, β-cubenene at 6.31 percent, β-cadinene at 6.16 percent, and α-bisabolol at 4.05 percent. In *I. pes-caprae*, the major constituent was (E)-caryophylene at 46.3 percent of the plant oils. Other major constituents included an unknown at 9.11 percent, α-humulene at 7.31 percent, and germacrene D at 7.62 percent. All the oil samples contained several minor constituents at less than five percent of the oil.

Several oil constituents were detected as minor amounts in the oil producing tissues, including pregeijerene (1.49%), 8-epi-dictamnol (1.21%), γ-muurolene (1.59%), and β-selinene (1.90%).

The brine shrimp lethality assay of the essential oils showed that *M. micrantha* essential oil (101.38 µg/mL) was the least active against the brine shrimp, although the essential oil of *I. pes-caprae* (92.50 µg/mL) also demonstrated limited bioactivity (Table 3). These observations contrast with the lethal activity of the essential oil from *C. odorata* (52.16 µg/mL).

DISCUSSION

The current study demonstrated differences in essential oils extracted from three Philippine vines. These differences in oil yield, oil color, and oil constituents could be affected by several factors, including the plant environment, plant genotype, and/or harvest maturity along with tissue variation (secretory cells and excretion cavities). Earlier research studies showed that chemical compositions of the essential oils of *C. odorata* from Cameroon and Congo mainly contain α-pinene and *p*-cymene (Lamaty, et al., 1992); while, from India, this plant contains pregeijerene, epi-cubebol, cubebol, *cis*-sabinene hydrate, germacrene-D-4-ol, α-cadinene, germacrene D, geijerene, cyperene, 10-epi-α-eudesmol, α-muurolol and khusimone (Joshi, 2013). The major oil constituents, such as α-pinene, geijerene, and pregeijerene, have been identified in *C. odorata* from Ivory Coast (Bedi, et al., 2001; 2004). Essential oil from *C. odorata* in Thailand contained pregeijerene,

germacrene D, α-pinene (Shimizu and Tomoo, 1994). The major of oil constituents in I pes-caprae in Mauritius were β-caryophene, 8-cedren-13-ol, and α-copaene. The essential oil of *Mikania micrantha* from Mexico contained linalool and α-pinene as the main components (Pérez-Amador et al., 2010).

Table 2. Chemical composition of essential oils from Philippine herbal vines.[1]

RI	Component	*C. odorata*	*M. micrantha*	*I. pes-caprae*	RI	Component	*C. odorata*	*M. micrantha*	*I. pes-caprae*
		----- Percentage composition-----					----- Percentage composition-----		
854	E-2 Hexenal	0.31±0.1	-	-	1499	Epi-cubebol	1.47±0.1	2.24±0.1	1.42±0.1
938	α-Pinene	0.69±0.2	-	-	1501	Bicyclogermacrene	1.14±0.1	1.76±0.1	0.45±0.1
1100	Linalool	0.39±0.1	-	-	1504	α-Muurolene	0.51±0.0	-	0.10±0.2
1130	α-Campholenal	0.35±0.1	-	-	1511	β-Bisabolene	0.81±0.4	2.59±0.3	0.47±0.2
1144	trans-Pinocarveol	0.92±0.1	-	-	1521	Cubebol	0.71±0.2	0.73±0.2	-
1146	Geijerene	0.35±0.0	-	-	1528	β-Cadinene	10.59±0.1	6.16±0.3	-
1146	Unknown	0.78±0.3	-	-	1532	Unknown	0.27±0.2	0.25±0.1	-
1156	Unknown	0.75±0.0	-	-	1534	Unknown	-	0.45±0.2	-
1168	Pinocarvone	0.58±0.1	-	-	1538	Trans-Cadina 1,4-diene	0.22±0.1	0.34±0.1	0.19±0.1
1171	Mentha-1,5-dien-8-ol	0.72±0.1	-	-	1546	α-Calacorene	0.5±0.0	-	0.44±0.1
1182	Terpin-4-ol	-	-	0.09±0.1	1552	Cis-cadinene ether	-	-	0.24±0.2
1193	α-Terpineol	0.16±0.4	-	0.08±0.1	1555	Unknown	-	0.94±0.1	-
1199	Myrtenal	0.66±0.1	-	-	1560	Unknown	-	0.59±0.2	0.33±0.2
1222	Cis-carveol	0.18±0.2	-	-	1564	Germacrene B	0.61±0.1	1.82±0.1	-
1257	Unknown	0.19±0.1	-	-	1566	(E)-Nerolidol	0.29±0.1	-	-
1289	Sabinyl acetate	0.53±0.2	-	-	1569	β-Calacorene	0.26±0.0	-	-
1294	Pregeijerene	1.49±0.1	-	-	1573	Unknown	2.71±0.2	-	-
1298	Unknown	-	-	0.18±0.1	1578	Spathulenol	0.59±0.1	2.98±0.1	0.44±0.1
1355	α-Cubebene	0.65±0.3	1.77±0.2	0.43±0.3	1585	Caryophyllene oxide	11.41±0.3	-	-
1372	Isoledene	0.12±0.0	-	-	1590	Unknown	0.18±0.4	6.71±0.0	9.11±0.2
1384	8-Epi-dictamnol	1.21±0.1	-	-	1594	Globulol	0.54±0.3	1.72±0.2	-
1388	Damascenone	-	0.23±0.1	0.39±0.2	1598	Carotol	2.43±0.1	0.29±0.1	0.47±0.2
1390	β-Bourbonene	0.43±0.1	-	-	1605	Cis-Dihydro mayurone	0.35±0.0	0.33±0.1	0.12±0.1
1394	β-Cubebene	0.52±0.1	6.31±0.2	2.22±0.2	1611	β–Atlantol	1.15±0.2	1.17±0.1	1.25±0.1
1400	β-Longipinene	0.68±0.2	-	-	1616	Junenol	0.98±0.0	0.95±0.0	-
1406	Unknown	-	0.27±0.1	-	1621	1,10-Di-epi-cubenol	1.29±0.2	1.31±0.1	0.28±0.2
1410	Unknown	0.54±0.2	-	-	1625	Unknown	0.39±0.2	0.35±0.2	0.16±0.1
1412	α-cis Bergamotene	-	-	0.12±0.1	1628	α-Corocalene	0.16±0.1	0.18±0.1	-
1419	β-Duprezianene	-	-	0.13±0.2	1629	Unknown	1.03±0.0	1.05±0.1	0.24±0.1
1426	(E)-Caryophyllene	10.90±0.1	2.19±0.1	46.3±0.2	1635	Cadinenol	2.30±0.2	2.32±0.2	1.17±0.3
1435	β-Copaene	0.53±0.4	0.13±0.2	0.22±0.1	1639	Eudesmol	0.15±0.0	0.17±0.1	0.59±0.1
1440	α-trans Bergamotene	1.99±0.6	0.46±0.2	0.12±0.1	1644	Allo-aromadendreneepoxide	0.89±0.0	-	-
1444	Unknown	0.25±0.3	-	-	1649	Cubenol	0.66±0.0	0.67±0.1	-
1448	z-β-Farnesene	-	0.06±0.1	-	1652	α–Muurolol	0.62±0.3	4.13±0.1	1.21±0.2
1452	Unknown	0.18±0.2	-	-	1662	Unknown	-	0.54±0.1	0.58±0.1
1457	trans-Muurola-3, 5-diene	0.51±0.3	0.44±0.1	-	1665	Epi-α-eudesmol	-	0.15±0.2	-
1460	α-Humulene	3.18±0.0	1.28±0.3	7.31±0.2	1668	Unknown	-	1.34±0.2	-
1464	Unknown	-	-	0.13±0.1	1671	Unknown	-	0.74±0.1	-
1466	β-Acordiene	-	0.16±0.1	-	1675	Unknown	-	1.34±0.4	-
1468	cis-Thujopsadiene	0.65±0.3	1.77±0.2	0.43±0.3	1682	Unknown	-	0.28±0.2	-
1473	Allo-aromadendrene	0.61±0.4	-	-	1685	Khusinol	-	1.13±0.1	0.61±0.3
1475	Cumacrene	-	0.27±0.1	-	1690	α-Bisabolol	-	4.05±0.3	-
1479	trans-Cadina-1(6), 4-diene	0.57±0.1	0.52±0.2	-	1692	Unknown	-	1.15±0.2	-
1481	γ-Muurolene	1.59±0.1	2.61±0.1	0.27±0.1	1704	Cis-thujopsenal	-	0.07±0.1	-
1487	Germacrene D	9.87±0.3	1.51±0.1	7.62±0.4	1732	(E)-Nuciferol	-	4.45±0.2	-
1489	β-Ionone	0.53±0.0	1.78±0.2	0.48±0.2	1796	epi-Cyclocolorenone	-	-	0.13±0.2
1492	β-Selinene	1.90±0.1	0.50±0.1	0.32±0.2					

[1]Each value in the table is represented as the mean ± SD; n = 3

Table 3. Lethal doses of essential oil Philippine herbal vines on brine shrimp larvae. .

Essential oil	Concentration (μg/mL)	Mortality (%)	Lethal Dose (LD_{50}, μg/mL)
Chromolaena odorata	1	17	52.16 ± 0.22
	10	30	
	100	77	
	1000	100	
Mikania micrantha	1	3	101.38 ± 0.35
	10	43	
	100	52	
	1000	100	
Ipomoea pes-caprae	1	3	92.50 ± 0.17
	10	17	
	100	53	
	1000	100	
Potassium dichromate	1	70	<1.0 ± 0.12
	10	100	
	100	100	
	1000	100	

The total number of surviving nauplii was the sum of triplicate determinations (n=3) with 10 nauplii in each trial.

The chemical composition of the essential oil often changes between different plant parts (Johnson, et al., 2004). Several factors affect essential oil composition, including the tissue variation (secretory cells and excretion cavities), the ontogenetic phase, and the growing location of the plant (Franz and Novak, 2010), such as topographical location, type of soil condition, and other environmental factors that impact plant growth and development. The volatile oil from the seeds and inflorescence of *Mikania micrantha* from Mexico contained linalool and α-pinene as the main components (Perez-Amador, et al., 2010), showing a completely different chemical composition as compared with the Philippine oils.

In contrast, only 22 compounds were detected and quantified from the research study on essential oils of the dried leaf samples of *M. micrantha* from China (Zhang, et al., 2004). The major oil compounds were identified as 2-butanamine (10.0%), β-longipinene (9.5%), β-humulene (7.8%), β-himachalene (7.1%), and curcumene (6.3%).

The chemical compositions of the essential oils of the dried leaves of *I. pes-caprae* from Philippines contained 32 compounds, representing 97.5% of the volatile compounds identified. The major compound was β-caryophyllene that dominated the profile of the oil (46.30%). As far as known, this is the first report of *I. pes-caprae* showing a high content of β-caryophyllene. Other major constituents were α-humulene (7.31%), germacrene D (7.62%), and caryophyllene oxide (9.11%). In the essential oil of *I. pes-caprae* from Mauritius, the major components were 8-cedren-13-ol (13.0%), (E)-nerolidol (7.0%), guaiol (6.2%), α-cadinol (6.2%) and limonene (6.1%) in fresh leaves and β-caryophyllene (36.6%), α-copaene (8.0%), germacrene D (7.3%), phytol (5.8%), δ-cadinene (5.7%), and α-humulene (5.4%) in the dried leaf samples (Marie, et al. 2007). These observations support the existences of chemotypes in this species as observed in other species (Juliani et al., 2006).

The brine shrimp lethality assay of the essential oils demonstrated that the oils showed some level of bioactivity. *M. micrantha* essential oil (101.38 μg/mL) was the least active against the brine shrimp, although the essential oil of *I. pes-caprae* (92.50 μg/mL) also demonstrated limited activity. These observations contrast with the activity of the essential oil from *C. odorata* which was lethal to 17% of the brine shrimp at 1 μg/mL. Volatile oil from leaves of *C. odorata* was cytotoxic against some intestinal pathogenic bacteria and demonstrated competitive inhibition extracellular protease of *Pseudomonas aeruginosa* (Adeola et al. 2015). Significant cytotoxicity against HeLa, HEp-2 and NIH 3T3 cancer cell lines has been reported (Prabhu et al., 2011).

Chemical components in the volatile oil from *M. micrantha* exhibited significant bioactivity on the ovipositioning of *Plutella xylostella*, *Phyllotreta striolata*, and *Phaedon brassicae*. The oil also possesses toxicity and reduces recover rates for *Lipaphis erysimi* (Zhang et al. 2004). The essential oil from *I. pes-caprae* has anti-inflammatory and antinociceptive activity (Marie et al., 2007). This action may be due to the presence of biologically active components in the oil, such as β-caryophyllene, phytol, and caryophyllene oxide in the dry leaves of *I. pes-caprae* (Tambe, et al. 1996; Pongprayoon et al. 1992).

As a whole, the brine shrimp lethality test of the essential oils from the Philippine herbal plants was concentration-dependent. The essential oil is biologically active when the LC_{50} values are less than 1000 μg/mL, while non-toxic or inactive when the LC_{50} values are greater than 1000 μg/mL (Meyer, et al. 1982). No reports on cytotoxic activity of essential oils in the leaves *C. odorata, M. micrantha, and I. pes-caprae* using the brine shrimp lethality assay have been observed. The level of bioactivity of essential oils could be due to the

concentration and the nature of the compounds present in plants.

The essential oils of guava (*Psidium guajava*) had more activity, exhibiting effects at 1 µg/mL (Fasola et al., 2011). The oil from hemp agrimony (*Eupatorium cannabinum* L.), was dominated by germacrene D and neryl acetate showed relatively high levels of bioactivity (16.3-22.0 µg/mL) (Judzentiene et al., 2016). Essential oils from cinnamon bark (*Cinnamomum zeylanicum*) and ginger rhizomes (*Zingiber officinale*) showed bioactivity at 10 and 0.03 µg/mL, respectively, against the brine shrimp (Sharififar et al., 2009). The brine shrimp appeared sensitive to a variety of essential oils. The diversity of the essential oil composition may be influenced by variations in climate and soil types that cause natural changes in plant growth that produce differences in the relative distribution of components in essential oils (Juliani et al. 2004; Tyler et al. 1976). As the essential oils were dominated mostly by sesquiterpenes, some of the oil components could be used as new sources of bioactivities with potential applications in the pharmaceutical, cosmetic and dietary supplement industries.

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