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Chemical Composition and Antioxidant DPPH Activity of the Floral and Leaves Essential Oils of *Montanoa speciosa* DC

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Abstract

The essential oils obtained by hydrodistillation from leaves and flowers of *Montanoa speciosa* collected in southeastern Mexico (Yucatan) were analyzed by GC-MS. A total of 71 and 79 components, representing 98.44% and 97.69% of the leaf and flower oils, respectively, were characterized. The main constituents found were β-caryophyllene (20.73%, 17.95%), δ-cadinene (9.88%, 9.28%), caryophyllene oxide (9.48%, 8.68%), and germacrene D (6.94%, 5.85%). The essential oils were screened for their antioxidant potentials by DPPH assay. The leaves oil exhibited higher DPPH scavenging capability (72.85 ± 0.28 mmol TE/g essential oil and 147.83 ± 0.41 mg/mL Vit C/g essential oil) than the floral oil (68.43 ± 0.10 mmol TE/g essential oil and 131.59 ± 0.87 mg/mL Vit C/g essential oil).

Keywords

Montanoa speciosa, Asteraceae, Essential Oil Composition, DPPH Assay

1. Introduction

Recent investigations in the field of antioxidants have focused on naturally occurring molecules to satisfy consumer concerns over safety and toxicity of food additives [1]. Antioxidants are both natural and synthetic compounds, able to scavenge free radicals and to inhibit oxidation processes [2]. Although, it was reported that synthetic antioxidants such as butyl hydroxytoluene (BHT), butyl hydroxyanisole (BHA), propyl gallate (PG), and tertiary butyl hydroquinone (TBHQ) have harmful effects in addition to their beneficial effects on food and

health [3]. A great number of aromatic, spicy, and medicinal plants contain chemical compounds, with antioxidant properties [4]. The antioxidant activity of plant extracts and essential oils is of particular interest because of their beneficial physiological activity on human cells and the potential they have to replace synthetic antioxidants used in foodstuffs [5] [6].

The *Montanoa* genus is one of the largest genuses belonging to the *Asteraceae* family. In México, there are 35 native species from this genus [7] [8]. Several species are of importance since they have been used in traditional medicine as abortive (*Montanoa tomentosa, M. grandiflora, M. frutescens*) and ornamental plants (*M. hibiscifolia, M. grandiflora*) [9] [10]. *M. tomentosa* known as "zoapatle" is a valued species due to its medicinal properties, mainly as a menstruation and childbirth inducer [11] [12].

Montanoa speciosa D. C., a species found in Yucatan, is a shrub about 2 m in height, with leaves and flowers very fragrant.

There are few reports concerning *Montanoa* essential oils. A study determined the chemical composition of essential oil from aerial parts of *Montanoa tomentosa* by SPME-GC-MS [13]. Another study assumes that the possible abortifacient activity is related to the chemical composition of essential oil from *Montanoa tomentosa* [14].

In this work, we describe, for the first time, the essential oil composition of *Montanoa speciosa* leaves and flowers using GC-MS and its antioxidant activity, evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

2. Material and Methods

2.1. Plant Material

Leaves and flowers of *Montanoa speciosa* DC (*Asteraceae*), were collected from a cultivated ornamental specimen in Merida, in the state of Yucatan, Mexico, in August and November of 2009, in the rainy season and flowering, respectively. The botanical identification of the specie was performed for one of the authors (L. Quijano).

2.2. Isolation of the Essential Oils

Dry leaves and flowers (50 g each one) of *M. speciosa* (**Figure 1**) were cut in small pieces and submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The oils were decanted and dried over anhydrous sodium sulphate.

The oil yield (calculated as oil w/w of dry extract) of leaf oil was 0.19%, and the floral oil was 0.59%. The oils were stored at 4˚C until their analysis.

2.3. Essential Oil Gas Chromatography-Mass Spectrometry Analysis

The oil samples were analyzed by gas chromatography-mass spectrometry, using Agilent technologies 6890 GC interfaced with a quadrupole mass spectrometer system 5973, and an Agilent Chemstation data system. The GC column was an HP-5MS fused silica capillary with a (5% phenyl)-methylpolysiloxane

(a) (b)

Figure 1. Leaves and flowers of *Montanoa speciosa*. (a) leaves; (b) flowers.

stationary phase (30 m × 0.25 μm d. i., 0.25 μm film thickness).

Inlet temperature and MSD detector temperatures were set at 270˚C and 290˚C, respectively. The GC oven temperature program was used as follows: from 60˚C - 246˚C at 3˚C/min and helium was employed as carrier gas (1 mL/min). The sample was dissolved in ethyl ether to give a 1% v/v solution. Injection size 1 μL using a split injection technique (split ratio 20:1) were used. MS were taken at 70 eV with mass range of m/z 41 - 300. Tetradecane (C14) as inner standard was used.

Identification of the components was achieved based on their lineal retention indices (KI, determined with reference to a homologous series to C_8-C_{20} *n*-alkanes) and by comparison of their mass spectral fragmentation patterns [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)] and comparing with literature data [15].

2.4. Determination of Antioxidant DPPH Activity

Radical scavenging activity of plant essential oils against to stable DPPH radical was determined spectrophotometrically [16] [17]. The colorimetric changes (from deep-violet to light-yellow) when DPPH is reduced chemically, were measured at 517 nm on a UV/visible light spectrophotometer. The antioxidant activities of the essential oils were measured in terms of hydrogen donation or radical scavenging ability (RSA).

Antioxidant capacity was expressed as the activity equivalent of two antioxidant compounds of reference Trolox and vitamin C. Calibration curve of DPPH solution (0.02 mg/mL) in methanol was performed to quantify the antioxidant activity of essential oil equivalent to Trolox (0.01 to 0.7 μg/mL) and vitamin C (01 - 20 μg/mL). Regression equation for each standard compound (Trolox and vitamin C) was calculated ($[DPPH] = (a \times Abs_{517}) + b$) to determine the equivalent concentration of each one. The absorbance of the solutions corresponding to each standard is read at a wavelength of 517 nm.

A volume of 750 μL of a methanolic solution (0.02 mg/mL) of essential oils were put into appropriate tubes, and 1.5 mL of 20 ppm methanolic solution of DPPH was added to each tube. Tests were carried out in triplicate. The decrease

in absorbance at 517 nm was determined after 15 min for all samples using a Perkin Elmer Lambda 11 spectrophotometer. Methanol was used to zero the spectrophotometer. Absorbance of the DPPH radical without antioxidant, *i.e.* the control, was measured.

Results are expressed in equivalent to the both standards used in the calibration activity (Trolox and vitamin C).

The radical scavenging capture percentage (% RSC) of the DPPH radical of each essential oil was calculated according to the formula [18]:

$$\%\ \mathrm{RSC} = \left(\frac{Ac - As}{Ac}\right) * 100$$

where % RSC = DPPH inhibition (%), Ac = absorbance of control sample and As = Absorbance of a tested sample.

3. Results and Discussion

Simple hydrodistillation of *M. speciosa* leaves and flowers produced a clear, colorless to pale yellow oils, with strong odor to wood.

The essential oils were analyzed by GC/MS for determination of their components and results are given in **Table 1** as a relative peak area of each constituent and their lineal retention index (LRI) values obtained on a HP-5MS capillary column. Typical GC chromatograms are presented in **Figure 2** and **Figure 3**.

In the essential oil from leaves 71 compounds were identified, corresponding to 98.4%, containing 93.4% of terpene derivates (5.4% and 88%, monoterpenes and sesquiterpenes, respectively). Sesquiterpenes hydrocarbons (60%) were prevalent to oxygenated sesquiterpenes (28.1%). Moreover among monoterpenes the hydrocarbonated species were also detected in a higher percentage (62.7%) than oxygenated (30.6%) (**Table 2**). The main constituents in *Montanoa speciosa* leaves essential oil were *β*-caryophyllene (20.7%), caryophyllene oxide (9.5%), germacrene D (6.9%), *α*-copaene (3.9%) and *δ*-selinene (3.7%).

In the essential oils of flowers, 79 compounds were identified. The monoterpenes constituted 10% and the sesquiterpenes constituted 84%, of which the hydrocarbonated sesquiterpenes had the most important contributions (54.4%). *β*-caryophyllene (17.9%), caryophyllene oxide (8.7%), germacrene D (5.8%), *α*-copaene (3.6%), *δ*-selinene (3.5%) and *α*-pinene (3.4%) were the major compounds in flowers essential oils.

The essential oils of *M. speciosa* showed that the major component was *β*-caryophyllene, being different with literature reports on the essential oils of the other *Montanoa* species in which the main compounds were monoterpenes [13] [19]. However, climatic, geographic conditions, environmental factors, vegetative cycle stage and type of extraction are among the reasons that could explain such differences [20].

Antioxidant activity of essential oils extracted by hydrodistillation from leaves and flowers of *M. speciosa* has been determined by one test system, namely, the DPPH assays. All data are presented in **Table 3**.

In the DPPH assay, the ability of the investigated essential oils to act as donors

Table 1. Chemical composition of floral and leaf essential oils from *Montanoa speciosa.*

Compound	LRI[a]	LRI[b]	Flower Area%	Leaf Area%	Compound	LRI[a]	LRI[b]	Flower Area%	Leaf Area %
Triciclene	903	921	0.1	tr[c]	*β*-Guaiene	1507	1502	1.9	2.0
α-Pinene	914	932	3.4	1.6	*α*-Farnesene	1513	1505	0.1	0.1
Camphene	931	946	1.5	0.8	Germacrene A	1515	1508	0.5	0.5
Sabinene	966	969	tr	-[d]	*δ*-Cadinene	1532	1522	9.3	9.9
β-Pinene	971	974	0.5	0.3	*α*-Cadinene	1540	1537	0.2	0.2
(*E*)-3-Octen-2-ol	977	982	0.2	0.2	*α*-Calacorene	1546	1544	0.1	0.1
NI[e]	994		0.1	0.1	Selin-3,7(11)-diene	1551	1545	0.7	0.5
p-Cymene	1022	1022	0.1	-	Elemol	1559	1548	0.4	0.4
Limonene	1024	1024	0.1	0.1	Germacrene B	1562	1559	0.2	0.3
1,8-Cineol	1024	1026	0.4	0.3	(*E*)-Nerolidol	1568	1561	0.1	-
cis-Sabinene hydrate	1057	1065	tr	tr	Longipinanol	1576	1567	0.2	0.2
Linalool	1086	1095	tr	tr	Germacren-D-4-ol	1581	1574	0.2	0.2
trans-Pinocarveol	1132	1135	0.1	0.2	Spathulenol	1587	1577	2.3	2.0
Camphor	1151	1141	1.6	1.7	Caryophyllene oxide	1592	1582	8.7	9.5
Borneol	1165	1165	0.2	0.2	Globulol	1603	1590	0.6	0.8
4-Terpineol	1178	1174	0.2	0.2	Viridiflorol	1605	1592	0.8	0.7
α-Terpineol	1184	1186	tr	-	Carotol	1614	1594	0.2	-
Bornyl acetate	1281	1284	1.7	1.7	Guaiol	1616	1600	0.4	0.3
α-Cubebene	1349	1345	0.3	0.3	Humulen-2-epóxide	1619	1608	0.4	0.5
α-Ylangene	1368	1373	0.3	0.3	*β*-Himanchalene oxide	1622	1615	0.6	0.6
Isoledene	1376	1374	0.1	0.1	NI	1624		0.2	-
α-Copaene	1378	1374	3.6	3.8	Dill apiole	1627	1620	0.2	0.2
β-Cubebene	1384	1387	0.1	0.1	1-*epi*-Cubenol	1632	1627	0.4	0.4
β-Bourbonene	1386	1387	0.3	0.4	*α*-Acorenol	1638	1632	0.3	0.2
β-Elemene	1392	1389	0.1	0.2	*cis*-Cadin-4-en-7-ol	1643	1635	1.8	1.8
β-Isocomene	1395	1407	0.5	0.8	*β*-Acorenol	1649	1636	0.7	0.6
z-Caryophyllene	1397	1408	0.1	0.2	*epi-α*-Cadinol	1651	1638	0.9	0.6
α-Cedrene	1414	1410	0.2	0.3	Caryophylla-4 (12), 8(13)-dien-5*α*-ol	1654	1639	1.3	1.2
β-Caryophyllene	1416	1417	17.9	20.7	allo-Aromadendrene epoxide	1657	1639	1.5	1.2
NI	1427		0.3	0.3	*epi-α*-Muurolol	1665	1640	2.3	2.5
cis-Muurola-3,5-diene	1449	1448	0.5	0.5	Cubenol	1668	1645	1.4	2.1
(*E*)-*β*-Farnesene	1452	1454	1.6	1.6	*β*-Eudesmol	1670	1649	0.6	-
***allo*-Aromadendrene**	1460	1458	0.4	0.3	*α*-Eudesmol	1673	1652	1.7	0.9
trans-Cadina-1(6), 4-diene	1471	1475	0.2	0.27	*trans*-Calamenen-10-ol	1684	1668	0.4	0.5
γ-Gurjunene	1477	1475	0.4	0.3	9-*epi*-(*E*)-caryophyllene-14-hydroxide	1686	1668	0.9	0.5
γ-Muurolene	1480	1478	1.0	0.9	*trans-α*-Bergamotol	0.4	0.3	1695	1690
Germacrene D	1485	1484	5.8	6.9	Farnesol	0.3	0.3	1700	1698
Aristolochene	1488	1487	2.4	2.7	NI	0.3	0.3	1705	
***β*-Selinene**	1496	1489	1.1	1.2	NI	0.6	0.3	1778	
***δ*-Selinene**	1499	1492	3.5	3.7					

[a]LRI determined experimentally. [b]LRI from reference. [c]Trace (<0.1%). [d]Not present. [e]Unidentified.

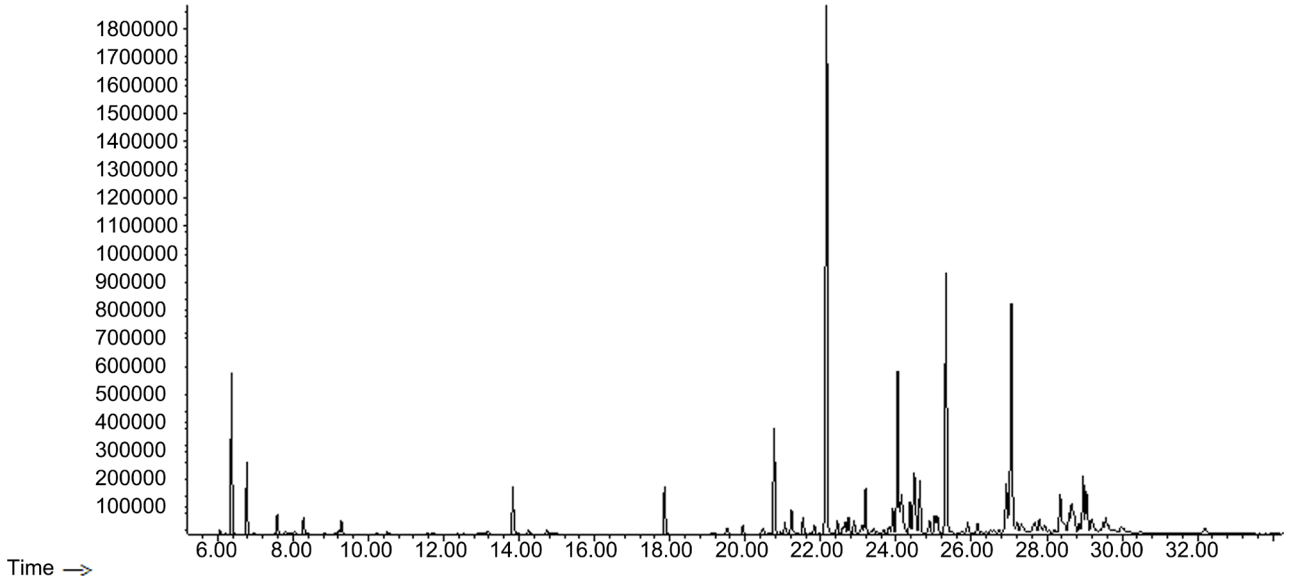

Figure 2. Typical GC chromatogram of essential oil of leaves of *Montanoa speciosa.*

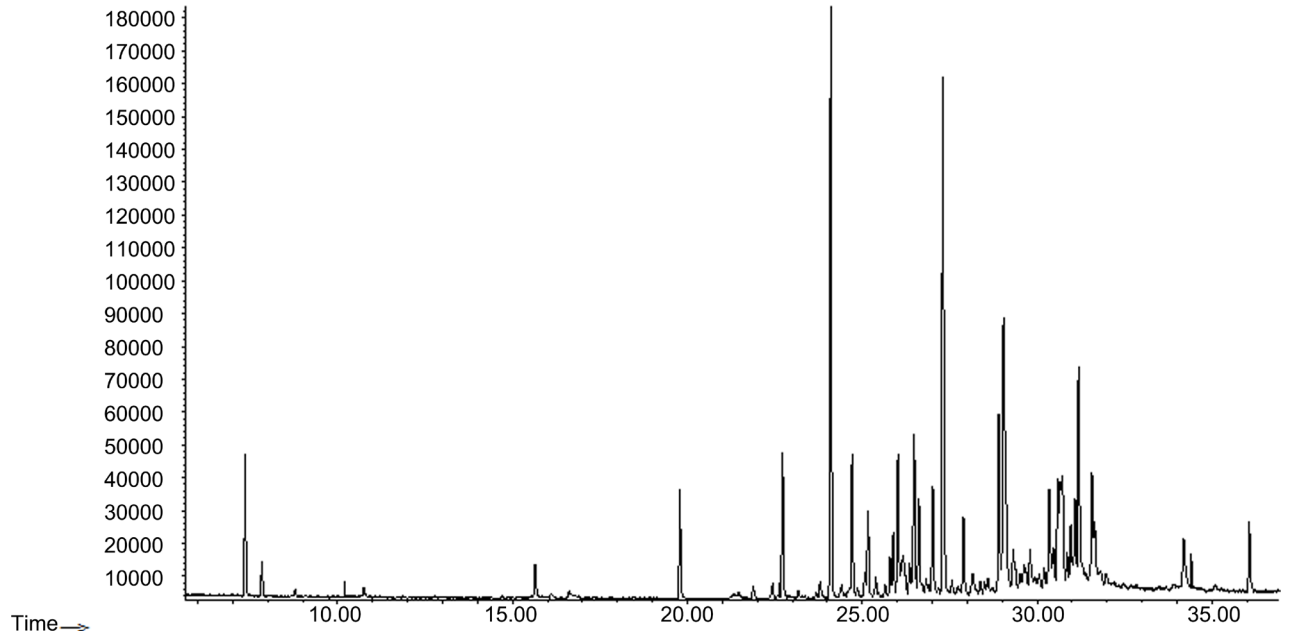

Figure 3. Typical GC chromatogram of essential oil of flowers of *Montanoa speciosa.*

Table 2. Area % of types of terpenes identified in essentials oil of leaves and flowers of *Montanoa speciosa.*

Grouped components	Leaf essential oil (%)	Floral essential oil (%)
Monoterpene hydrocarbons	2.8	5.6
Oxygen-containing monoterpenes	2.6	4.4
Sesquiterpene hydrocarbons	59.9	54.4
Oxygen-containing sesquiterpenes	28.0	29.6
Others	3.2	3.6

Table 3. Radical scavenging capture percentage (% RSC) of *M. speciosa* essential oils and Vit C and Trolox.

Sample[a]	% RSC
Leaves	65.4170 ± 0.193
flowers	61.3616 ± 0.061
Vit C	97.5317 ± 0.059
Trolox	41.1212 ± 0.597

[a]Concentrations: Vitamin C 20 ppm, Trolox 50 ppm and essential oils 40 ppm.

of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was investigated. All of the assessed essential oils were able to reduce the stable, purple-colored radical DPPH to yellow colored DPPH-H.

According to DPPH assay the essential oils of the two parts of *M. speciosa* showed moderate radical scavenging capture percentage (% RSC) respect to vitamin C, but a greater activity compared with Trolox. The essential oil of leaves showed higher activity compared with the floral essential oil.

The antioxidant capacity expressed in terms of equivalent of Trolox (TE) and vitamin C, is showed in **Table 4**.

As showed, the antioxidant capacity of the leaves essential oil presented an activity slightly higher than the flowers essential oils.

There are not previous reports about % RSC or antioxidant capacity of *Montanoa speciosa*, or other *Montanoa* species essential oils.

In literature revised there are no reports related to the chemical composition of the essential oils of *Montanoa speciosa* DC, however, there are reports of the study of the essential oils of *Montanoa tomentosa*, a plant used by indigenous populations in the center of the country as an abortifacient. Compadre *et al.* [14] performed the GC-MS analysis of the leaves and found that the major compounds were bornyl acetate, β-cubebene and β-caryophyllene. While, Robles *et al.* [13] performed the analysis of the chemical composition of leaves and flowers of *Montanoa tomentosa* by SPME-CG-EM and found in both cases that more than 65% of the compounds belong to the monoterpene series, being the compounds sabinene, α-pinene and α-thujene, the majority; In contrast to what was observed in this study. From *Montanoa speciosa* there are reports of biological activity and structural elucidation of sesquiterpene lactones obtained from the aerial parts of this plant [21], Sabanero *et al.* [22], Quijano *et al.* [23].

4. Conclusion

In the present investigation, chemical composition and scavenging activity with DPPH assay of essential oils of dry leaves and flowers of *M. speciosa* were evaluated. β-Cayophyllene, δ-cadinene, caryophyllene oxide and germacrene D were the major constituents. With reference to oil composition, a large difference can be observed comparing to the sample analyzed in Mexico for plants of same genus. However, the composition is similar between different organs of the same plant with few variations, which may be due to the harvest season. About

Table 4. Antioxidant capacity of the different parts of *M. speciosa* essential oil.

Part of Plant	Antioxidant DPPH Capacity
Leaves	72.85 ± 0.28 mmol TE/g essential oil 147.83 ± 0.41 mg/mL Vit C/g essential oil
Flowers	68.43 ± 0.10 mmol TE/g essential oil 131.59 ± 0.87 mg/mL Vit C/g essential oil

activity antioxidant, the capacity of the leaves essential oil presented an activity slightly higher than the flower essential oil.

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