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Zaluzania montagnifolia: essential oil composition and biological properties

[*Zaluzania montagnifolia*: composición del aceite esencial y actividades biológicas]

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Abstract: The chemical composition of the seasonal essential oils (2015-2016) from the leaves and flowers of *Zaluzania montagnifolia* is presented. The chemical content of those oils showed quantitative and qualitative differences. Germacrene D (19.9-29.8%), camphor (12.4-19.4%) and β-caryophyllene (13.7-18.5%) were the most abundant volatiles in the leaves. The essential oils from the flowers contained high amounts of camphor (32.7-37.2%) limonene (19.8-24.9%) and germacrene D (3.2-7.3%). All the seasonal essential oils showed a potent *in vitro* inhibition against HMG-CoA reductase. The essential oils from flowers (IC_{50}, 40.5-55.1 µg mL^{-1}) showed better inhibition properties than those of leaves (IC_{50}, 84.4-123.5 µg mL^{-1}). Camphor (IC_{50}, 72.5 µg mL^{-1}) and borneol (IC_{50}, 84.4 µg mL^{-1}) exerted a non-competitive inhibition on the enzyme. Additionally, the hydrodistillates exhibited antibacterial activity against the phytopathogenic *Pseudomonas syringae* pv. *tabaci* TBR2004 (MIC, 62.7-76.5 µg mL^{-1}) *P. syringae* pv. *tomat*o DC3000 (MIC, 45.4-50.4 µg mL^{-1}) and *P. syringae* pv. *phaseolicola* NPS3121 (MIC, 26.7-31.9 µg mL^{-1}). Germacrene D (MIC, 35.4-66.2 µg mL^{-1}) and β-caryophyllene (MIC, 36.5-54.2 µg mL^{-1}) were the strongest anti-*Pseudomonas syringae* agents.

Keywords: *Zaluzania montagnifolia*, seasonal essential oils, chemical profile, anti-HMG-CoA reductase, antibacterial.

Resumen: Se presenta la composición química de los aceites esenciales estacionales (2015-2016) provenientes de hojas y flores de *Zaluzania montagnifolia*. El contenido químico de los aceites esenciales mostró diferencias cualitativas y cuantitativas. El germacreno D (19.9-29.8%), alcanfor (12.4-19.4%) y β-cariofileno (13.7-18.5%) fueron los volátiles más abundantes en las hojas. Los aceites esenciales de las flores contuvieron altas concentraciones de alcanfor (32.7-37.2%), limoneno (19.8-24.9%) y germacreno D (3.2-7.3%). Todos los aceites esenciales estacionales mostraron una potente inhibición *in vitro* contra la HMG-CoA reductasa. Los aceites esenciales de las flores (IC_{50}, 40.5-55.1 µg mL^{-1}) mostraron mejores propiedades inhibitorias que aquellos de las hojas (IC_{50}, 84.4-123.5 µg mL^{-1}). El alcanfor (IC_{50}, 72.5 µg mL^{-1}) y el borneol (IC_{50}, 84.4 µg mL^{-1}) ejercieron una inhibición no competitiva sobre la enzima. Adicionalmente, los hidrodestilados exhibieron una actividad antibacterial contra los fitopatógenos *Pseudomonas syringae* pv. *tabaci* TBR2004 (MIC, 62.7-76.5 µg mL^{-1}) *P. syringae* pv. *tomato* DC3000 (MIC, 45.4-50.4 µg mL^{-1}) y *P. syringae* pv. *phaseolicol*a NPS3121 (MIC, 26.7-31.9 µg mL^{-1}). El germacreno D (MIC, 35.4-66.2 µg mL^{-1}) y β-cariofileno (MIC, 36.5-54.2 µg mL^{-1}) fueron los agentes más fuertes contra los patovares de *Pseudomonas syringae*.

Palabras clave: *Zaluzania montagnifolia*, aceites esenciales estacionales, perfil químico, anti-HMG-CoA reductasa, antibacterial.

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INTRODUCTION

The roots of *Zaluzania montagnifolia* (Asteraceae) are traditionally boiled to prepare an infusion for treating type 2 diabetes mellitus (DM2) in Oaxaca, México (Turner, 2012). Previous reports described the aerial parts of the plant as a source of sesquiterpene lactones (zaluzanins) and kaurene type diterpenes with hypoglycemic, cytotoxic and uterotonic properties (Krishna-Kumari *et al*., 2003). Kaurenoids and other small molecules dissolved in the hexanic fraction of the plant also exhibited allelopathic, antioxidant and antibacterial activities (Villa-Ruano *et al*., 2013). Despite the pleasant aroma of the leaves and flowers of *Z. montagnifolia*, there is no available information on the chemical composition of its essential oil. Essential oils from medicinal/edible plants are a potential source of small molecules with pharmacological activity. The essential oils from *Lippia turbinata*, *Origanum vulgare* and *Salmea scandens* showed interesting *in vitro* activity against fat absorption (Quiroga *et al*., 2013; Villa-Ruano *et al*., 2015a). These facts suggest that natural products from medicinal plants could be used in the modulation of fat metabolism. Some volatile organic compounds such as geraniol and limonene were apparently able to decrease the activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), a key enzyme in cholesterol biosynthesis (Pattanayak *et al*., 2009). Thus, volatiles from medicinal plants can exert an improvement in the metabolism of cholesterol. Natural inhibitors of cholesterol biosynthesis are found in traditional plants and their inclusion as an alternative for the treatment of hypercholesterolemia is being envisioned (Lin *et al.*, 2015). It is well known that high levels of LDL-cholesterol particles are a risk factor for the development of DM2 and also that patients with DM2 maintain those levels when they are incorrectly treated (Mooradian, 2009). Therefore, the investigation of natural products from medicinal plants with potential use as inhibitors of HMG-CoA reductase should be investigated.

On the other hand, alternative biological activities of medicinal plant must be explored. Antibacterial activity of essential oils has been extensively demonstrated in pathogens of human, however, the effect of these preparations against phytopathogenic bacteria has been poorly investigated (Villa-Ruano *et al*., 2015a; 2015b). *P. syringae* pathovars are one of the most prevalent microorganisms causing the bacterial brown spot and halo blight diseases in *Phaseolus vulgaris*, *Lycopersicon esculentum*, *Nicotiana tabacum* and *Solanum tuberosum* (González *et al*., 2000) which are considered as basic crops in rural communities in México. Thus, plants with traditional uses in this communities can be screened for their anti-phytobacterial properties in order to propose alternatives for the biological control of phytophatogens. Due to the lack of scientific information regarding the volatile profile and biological activities of the essential oil from *Z. montagnifolia*, the principal objectives of this work were to report the volatile variation in the essential oil from the plant and to determine the specific effect of selected volatiles on the normal activity of HMG-CoA reductase. We additionally report on the antibacterial effect of the hydrodistillates on some *Pseudomonas syringae* pathovars which are associated to severe infections of potato, tomato and bean.

MATERIALS AND METHODS

Plant material

Z. montagnifolia (SCH. BIP.) SCH. BIP. (Asteraceae), was collected in Miahuatlán de Porfírio Díaz, Oaxaca, México (16° 20.63' N, 0.96° 34.85' W, 1580 masl) during 2015-2016. Aerial parts of the assayed plants were previously certified in the FCME-Herbarium at UNAM-México where a reference voucher 130910 was deposited.

Chemicals

Analytical grade *n*-hexane was purchased from J.T Baker. Anhydrous sodium sulfate was from Bell Reactives^{MR}. The HMG-CoA reductase enzyme, NADPH, 3-hydroxy-3-methylglutaryl CoA, resazurin, mixtures of *n*-alkanes and authentic volatiles were all from Sigma-Aldrich Co.

Isolation of the essential oils

Leaves of *Z. montagnifolia* were collected and dried in an oven at 30° C for 3 days in darkness. Seasonal leaf samples were collected in spring (March 2015), summer (July 2015), fall (October 2015), winter (January 2015) and spring 2016 (March 2016). Seasonal flower samples were only collected in the spring periods, during March 2015 and March 2016. 300 g of dried leaves from five different plant samples were extracted by hydrodistillation (five for each season, n=5) in a Clevenger type apparatus for 3 h in order to obtain five different essential oil

samples. Posteriorly, the essential oils were recovered with *n*-hexane and immediately dried over anhydrous sodium sulfate. The density (mg mL^{-1}) and yields of the essential oils (w/w) were obtained and stock solutions (200 mg mL^{-1}) were prepared to evaluate the hydrodistillates on HMG-CoA reductase and to perform antibacterial tests (Villa-Ruano *et al.*, 2015a).

GC-FID and GC-MS analyses

These procedures were done in a Hewlett Packard 6890 II series with a HP-5 capillary column (30m X 0.25mm I.D. covered with a 0.25 µm of 5:95 phenyl-dimethylpolysiloxane as stationary phase). The GC-MS data were additionally corroborated in a Varian CP3800 gas chromatograph equipped with a capillary column Factor Four VF-5ms (30m X 0.25mm I.D, 25 µm of 5:95 phenyl-dimethylpolysiloxane as stationary phase) coupled to a Varian quadrupole 320MS model. The mobile phase was helium at 1 mL min^{-1} flow rate. The GC-FID and GC-MS run conditions were adjusted in accordance with the analytical procedures of a previous report (Villa-Ruano *et al*., 2015b). Metabolites were identified by comparison of their mass spectra (70 eV) with those of the NIST 2.0 Standard Reference Database, with the literature (Adams, 2007) and by co-injection with available authentic standards. Kovats index (RI) was calculated by simultaneous runs using a mixture of *n*-alkanes (C_8-C_{20}, C_{21}-C_{40}). The abundance of the metabolites was calculated on the basis of GC-FID peaks and the variation of the main volatiles was validated by ANOVA-Tukey Tests ($p<0.01$).

Anti-HMG-CoA reductase activity

This activity was determined in each seasonal essential oil (0.02-0.2 mg mL^{-1}) by the spectrophotometric assays of NADPH oxidation described by Gholamhoseinian et al. (2010), with slight modifications. The buffer described by those authors was replaced by Tris-EDTA (0.1 M, pH 7.4) and Pravastatin (Merck ®) was used as standard of inhibition. The IC_{50} was calculated from five essential oils per season, then it was averaged and presented as IC_{50} ± SD. The specific kind of inhibition of the essential oils, camphor and borneol was determined by double reciprocal Lineweaver-Burk plot regressions performed with the GraphPad Prims 5 software. The regressions were carried out using distinct concentrations of HMG-CoA (0.1 - 2 mmol L^{-1}) and three concentrations of the essential oils or pure volatiles (0.02, 0.08 and 0.14 mg mL^{-1})

Antibacterial activity

The effect of the essential oils on the *in vitro* growth of *Pseudomonas syringae* pv. *tabaci* TBR2004, *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *phaseolicola* NPS3121, was investigated by the broth microdilution method using rezasurin as an indicator of cell viability (Sarker *et al*., 2007). The incubations were performed with different concentrations of the essential oil or authentic standards of germacrene D, camphor, limonene and β-caryophyllene (10-500 µg mL^{-1}) with $5X10^5$ UFC in a 96-well plate in quintuplicate at a final volume of 0.3 mL. Dose-response curves (10-200 µg mL^{-1}) were performed with Agrigen Plus® as a standard of reference. Absorbance was measured at 545 nm. The MIC value was considered as the initial concentration able to avoid any absorbance change in the dose response curves. The MIC values were presented as the average of the five essential oil samples obtained in each season studied.

RESULTS AND DISCUSSION

The yields of the yellow crystalline essential oils from *Z. montagnifolia* was estimated in the range of 3.5-4.2% (w/w) for the analyzed samples. Thirty-two known compounds were identified in the essential oils from the flowers and leaves of *Zaluzania montagnifolia* (Table No 1). The abundance of sesquiterpenes in the leaves was higher (>50%) than that of monoterpenes in the seasons studied (~40%). In those hydrodistillates, germacrene D (19.9-29.8%) was the majoritarian sesquiterpene followed by camphor (12.4-19.4%) and β-caryophyllene (13.7-18.5%). Significant fluctuations ($p<0.01$) in the relative abundance of germacrene D in leaves were observed along the analyzed seasons but, the abundance of the other two majoritarian volatiles was not significantly changed (Figure No 1). Contrary to the essential oils from leaves, those from flowers showed a higher abundance of monoterpenes (~80%) than sesquiterpenes (~18%) (Table No 1). In this case, camphor (32.7-37.2%), limonene (19.8-24.9 %) and germacrene D (3.2-7.3%) were the most abundant volatiles. In the two flowering seasons studied (spring 2015 and spring 2016), the three main volatiles showed significant changes ($p<0.01$) (Figure No 2). The abundance of camphor is usually conferred to leaves, shoots, stem barks, roots or rhizomes of many plants but, its presence in flowers

is has been less reported (Mojab *et al*., 2007). Dramatic differences in the chemical composition between the analyzed essential oils and the hexanic fractions from *Z. montagnifolia* were observed (Villa-Ruano *et al*., 2013). According to these results the essential oil of the plant could be considered as an alternative source for the obtainment of camphor and germacrene D.

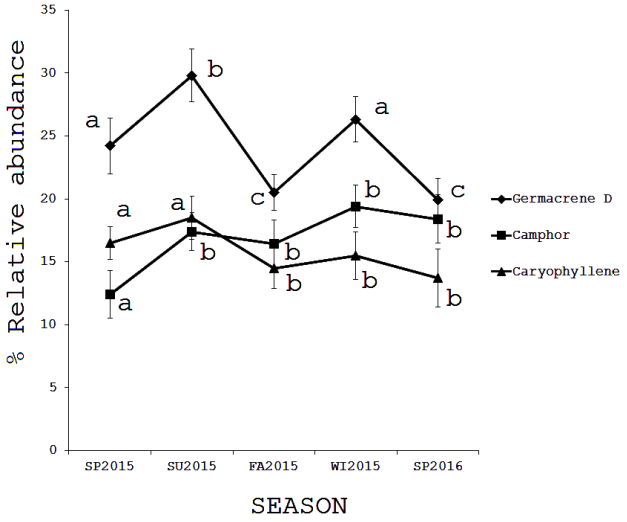

Figure No 1
Chemical variation of germacrene D, camphor and β-caryophyllene in the leaves of *Zaluzania montagnifolia*. Bars represent the standard deviation of five different seasonal samples. Diverse letters indicate statistically significant differences between means (p<0.01).

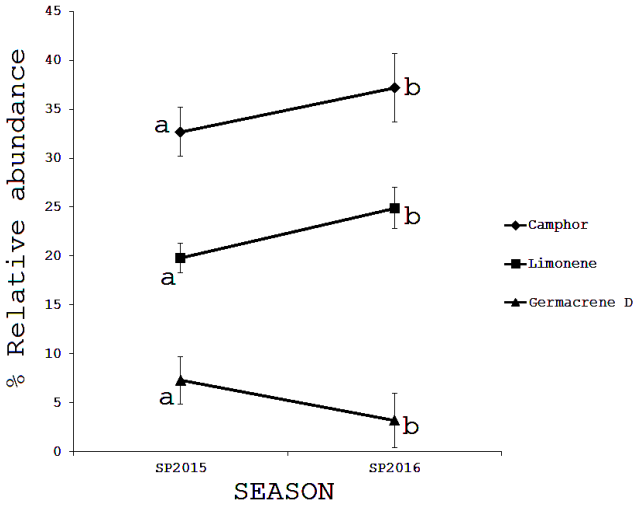

Figure No 2
Chemical variation of camphor, limonene and germacrene D in the flowers of *Zaluzania montagnifolia*. Bars represent the standard deviation of five different seasonal samples. Diverse letters indicate statistically significant differences between means (p<0.01).

Table No 1
Chemical composition of the seasonal essential oils (2015-2016) from the leaves and flowers of *Zaluzania montagnifolia*.

	Leaves (%)							Flowers (%)	
Compound	[c]SP2015	[c]SU2015	[c]FA2015	[c]WI2015	[c]SP2016	RI1[b]	RI2[b]	[c]SP2015	[c]SP2016
[a]nonane	0.3±0.08	0.9±0.12	-	-	0.7±0.05	900	900	0.4±0.06	0.9±0.11
Tricyclene	-	-	0.3±0.04	1.5±0.18	0.9±0.12	923	921	0.3±0.05	-
α-Thujene	-	-	0.7±0.02	1.3±0.05	0.6±0.08	931	933	0.3±0.07	-
[a]α-Pinene	0.5±0.06	1.4±0.10	-	0.7±0.05	1.3±0.16	935	935	3.6±0.41	0.9±0.12
Camphene	0.7±0.02	2.8±0.34	-	1.5±0.17	2.6±0.05	952	954	3.8±0.46	0.6±0.04
Sabinene	0.8±0.13	-	0.6±0.02	0.9±0.12	-	971	973	-	-
[a]β-Pinene	0.4±0.07	-	-	-	1.8±0.23	978	978	1.9±0.22	4.2±0.31
1-Octen-3-ol	0.7±0.09	2.6±0.17	-	-	-	980	981	2.8±0.31	3.1±0.39
β-Myrcene	0.6±0.04	-	0.3±0.04	-	-	994	995	0.8±0.14	0.6±0.08
[a]Limonene	1.5±0.18	3.6±0.43	7.6±0.59	4.8±0.05	0.9±0.06	1030	1032	19.8±2.1	24.9±2.95
Ocimene	0.5±0.04	0.5±0.03	-	-	1.1±0.14	1043	1044	0.6±0.08	-
α-Terpinene	-	-	0.8±0.12	0.8±0.11	0.6±0.08	1063	1064	0.4±0.05	-
Linalool oxide	0.3±0.08	2.8±0.06	-	-	1.7±1.32	1074	1073	0.9±0.05	1.5±0.17
Terpinolene	-	-	0.4±0.06	0.4±0.05	0.8±0.09	1085	1086	0.6±0.08	-
[a]Linalool	1.4±0.17	4.7±0.49	-	3.1±0.05	2.2±0.24	1098	1099	1.9±0.22	2.4±0.29
[a]Camphor	12.4±0.92	17.4±2.12	16.4±1.57	19.4±0.22	18.4±0.05	1138	1139	32.7±3.9	37.2±4.33
[a]Borneol	10.4±0.74	4.4±0.51	11.9±0.12	3.4±0.05	9.7±1.12	1166	1167	3.5±0.39	4.5±0.48
p-menth-1-en-8-ol	1.3±0.05	-	-	0.3±0.02	-	1189	1187	0.8±0.09	1.3±0.15
Copaene	0.4±0.07	0.2±0.02	0.9±0.13	-	-	1375	1376	0.8±0.11	-
β-Cubebene	0.7±0.09	0.5±0.07	-	-	0.3±0.05	1390	1391	-	0.7±0.04
β-Caryophyllene	16.5±1.8	18.5±1.52	14.5±1.23	15.5±0.14	13.7±1.56	1418	1417	2.4±0.25	3.4±0.38
Humulene	4.2±0.53	0.5±0.09	4.7±0.15	2.2±0.25	0.9±0.12	1449	1450	-	0.9±0.08
[a]Germacrene D	24.2±1.6	29.8±3.4	20.5±.1.8	26.3±2.9	19.9±0.05	1482	1483	7.3±0.85	3.2±0.37
α-Selinene	3.2±0.43	0.9±0.05	2.6±0.39	1.1±0.09	2.5±0.24	1489	1490	2.6±0.20	-
Cadina-1(10),4-diene	0.5±0.07	-	-	0.9±0.07	-	1524	1525	0.6±0.07	-
Nerolidol	0.5±0.09	-	-	-	-	1533	1533	0.2±0.05	0.8±0.09
Spathulenol	1.9±0.15	0.3±0.05	0.8±0.05	0.7±0.09	0.5±0.03	1571	1571	0.5±0.09	-
Germacrene D-4-ol	6.2±0.90	1.2±0.05	3.2±0.22	9.4±1.1	4.1±0.42	1575	1576	2.1±0.25	3.1±0.39
tau.-Cadinol	1.2±0.09	0.9±0.03	2.6±0.34	-	1.7±0.15	1628	1629	0.4±0.01	-
α-Bisabolol	1.4±0.08	0.4±0.06	0.4±0.06	-	0.7±0.02	1684	1684	0.6±0.09	0.3±0.04
Epimanool	2.6±0.07	0.6±0.08	3.1±0.43	3.4±0.21	3.1±0.37	1961	1962	2.5±0.29	3.5±0.41
Phytol	2.5±0.33	0.7±0.07	5.7±0.65	1.2±0.17	3.7±0.43	2112	2113	-	
Monoterpenes	31.5	40.2	39	38.1	42.6			74.7	81.2
Sesquiterpenes	60.9	53.2	50.2	56.1	44.3			17.5	12.4
Diterpenes	5.1	1.3	8.8	4.6	6.8			2.5	3.5
Hydrocarbons	0.3	0.9	0	0	0.7			0.4	0.9

Total	97.8	95.6	98	98.8	94.4		95.1	98

[a] Compounds identified by co-injection with authentic standards.
[b] RI1 and RI2, retention indices for HP-5ms and Factor Four VF-5ms columns, respectively.
[c] SP spring; SU; summer; FA, fall; WI, winter. A dash (-) indicates no component identified

Table No 2
Anti-HMG-CoA activity of the seasonal essential oils from *Zaluzania montagnifolia*

[a]Chemical agent	[b]SP2015	[b]SU2015	[b]FA2015	[b]WI2015	[b]SP2016
Leaf essential oil	94.5±8.7	104.5±.9.2	115.4±6.5	84.4±7.8	123.5±9.7
Flower essential oil	55.1±4.5	-	-	-	40.5±3.9
Camphor			72.5±2.9		
Borneol			84.4±2.6		
Pravastatin			0.12±0.002		

(IC_{50}±SD)
[a]IC_{50} values are expressed in µg mL^{-1}.
[b]SP, spring; SU, summer; FA, Fall; WI, winter.

The seasonal essential oils from the leaves decreased the activity of the enzyme using approximately 0.1 mg mL^{-1} (Table No 2). However, slight differences in the IC_{50} value were perceived in the essential oils of spring 2015 and winter 2015 (Table No 2). On the other hand, the essential oils from the flowers (IC_{50}, 40.5-55.1 µg mL^{-1}) displayed a better inhibition rate than those of leaves (IC_{50}, 84.4-123.5 µg mL^{-1}). Under standard conditions the K_m of HMG-CoA for HMG-CoA reductase was 0.532 mM and the V_{max} was 0.0116 mM min^{-1}. The essential oils from both aerial organs produced a non-competitive effect on HMG-CoA reductase (Figure No 3 A-B). V_{max} was reduced from 0.0116 to 0.0081 mM min^{-1}, whereas the K_m parameter was not affected (0.531 mM). The chemical profiles of the essential oils from the flowers, revealed camphor as the main constituent (Table No 1). The enzymatic test with pure camphor suggested that this compound was strongly involved in the *in vitro* inhibition of the enzyme (Table No 2). Camphor reduced V_{max} from 0.0116 to 0.0065 mM min^{-1} (Figure No 3C), whereas the K_m value was not significantly changed (0.530 mM). Interestingly, no inhibitory effects on HMG-CoA reductase were observed for germacrene D or β-caryophyllene at the assayed concentrations (<0.2 mg mL^{-1}). This fact strongly suggested that other compounds in the essential oils were also participating to exert the inhibitory activity. Borneol (3.4-10.4%) was tested for the same activity observing its effect as an inhibitor of the studied enzyme (Table No 2). As is well known, this compound is structurally related to camphor. Borneol was able to decrease V_{max} to 0.0085 mM min^{-1} (Figure No 3D). As non-competitive inhibitors, camphor and borneol are able to cause a delay in the production of cholesterol intermediates, thus the final production of the triterpene will be decreased as the inhibitor interacts with the enzyme. Remarkably, camphor and its isomer borneol have been used as flavoring agents found in some plant foods such as basil, coriander, marjoram, rosemary and sage which are used since ancient times (Aguilar *et al*., 2008). Previous reports revealed that other monoterpenes such as limonene exerted a potent inhibitory effect on the studied enzyme (Pattanayak *et al*., 2009). However, this is the first work reporting the inhibitory effect of oxygenated camphor and borneol on the assayed enzyme. In spite of the potential toxicity of these oxygenated monoterpenes (Aguilar *et al*., 2008), the oral administration of small quantities of camphor and borneol in rats (<50 mg $Kg^{-1,}$ bw) demonstrated that these compounds appeared in plasma after 2 hours (Sun *et al*., 2014). These studies additionally suggested that camphor can be rapidly converted into borneol, a less toxic compound. Thus, the presence of these volatiles in rat plasma strongly suggest their bioavailability to interact with different physiological targets. Recently, it was reported that the essential oils from ginger and rosemary exerted a hypocholesterolemic effect in rats (Eissa *et al*., 2017). These oils contained camphor and borneol as

majoritarian volatiles. In accordance with our results and previous literature, the strongest effect of the essential oils from the flowers of *Z. montagnifolia* could be linked to the synergistic effect of limonene, camphor and borneol. Further *in silico* approximations should be required to predict the interaction of those volatiles with HMG-CoA reductase.

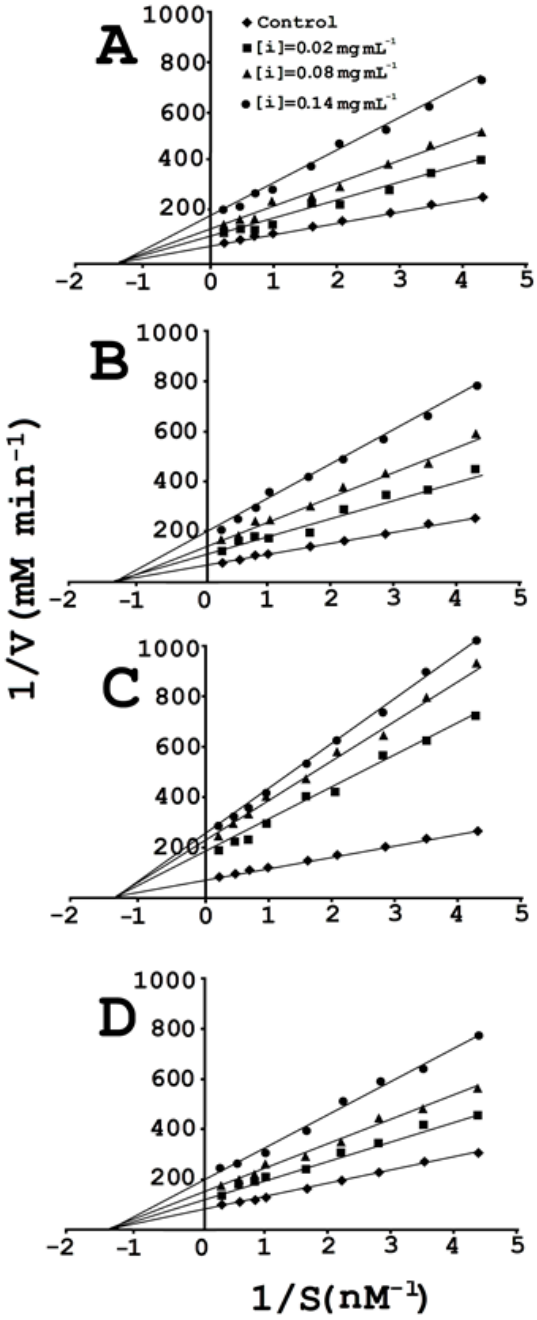

Figure No 3
Lineweaver-Burk plot regressions of the essential oils from *Z. montagnifolia* on the specific activity of HMG-CoA reductase. A, effect of the essential oil from leaves (Spring 2016). B, effect of the essential oil from the flowers (Spring 2016). C, effect of authentic camphor. D, effect of authentic borneol. The concentrations of HMG-CoA was in the range of 0.1-2 mmol L^{-1}

The antibacterial activity of the essential oils was observed for the three assayed *Pseudomonas syringae* pathovars. However, the seasonal essential oils were more effective on the growth inhibition of the pathovar phaseolicola (Table No 3). The IC_{50} values were relatively higher for the pathovars tomato and tabaci but, according to our results the less affected bacterial strain was the pathovar tabaci (Table No 3). In all the cases, the commercially available antibiotics were more effective than the assayed essential oils (Table 3). Nevertheless, the use of these oils could be envisioned as an alternative for the biological control of those phytopathogenic microorganisms. The hydrodistillates from *Z. montagnifolia* showed a better *in vitro* inhibitory effect than the essential oils from *Clinopodium macrostemum* and *Salmea scandens* in the same bacterial species (Villa-Ruano *et al.*, 2015a; 2015b). Interestingly, the assessment of the majoritarian terpenes revealed that camphor was not completely involved in the anti-bacterial activity (Table No 4). The high MIC value of camphor and limonene (~ 0.3 mg mL^{-1}), suggested that these monoterpenes produced a slight toxicity in the three pathovars of *Pseudomonas syringae*. This property has been observed in *Pseudomonas putida* which is able to convert camphor and limonene into less aggressive derivatives (Prasad *et al.*, 2011; Loeschcke & Thies, 2015). Controversially, *P. putida* shows slight sensitivity to the action of camphor but not to that of its isomer borneol (Prasad *et al.*, 2011). These evidences suggest that the assayed bacterial strains could probably contain a similar biochemical biotransformation mechanisms to that of *Pseudomonas putida* (Prasad *et al.*, 2011; Loeschetcke & Thies., 2015). Germacrene D and β-caryophyllene showed the best antibacterial activity in the three assayed pathovars (Table 4). The growth inhibitory properties of β-caryophyllene has been already demonstrated in *Pseudomonas syringae* pv. tomato (Huang *et al.*, 2012). However, up to our knowledge this is the first report on the antibacterial activity of germacrene D on *P. syringae* pathovars. According to these evidences, our results suggest that the effect of the assayed essential oils is strongly related to the abundance and synergistic activity of several volatiles dissolved these hydrophobic fractions. This finding could be considered as the initial approach for further *in vivo* assays with diseased plants. Considering that these bacterial species are a threat for crops and for farmers's economy, the obtainment of new alternatives for their biological control of *P. syringae* would be very valuable as a contribution to resolve this problem (González *et al.*, 2000).

Table 3
Antibacterial activity of the seasonal essential oils from *Zaluzania montagnifolia* (MIC±SD).

[***]Bacteria	[b]SP2015	[b]SU2015	[b]FA2015	[b]WI2015	[b]SP2016	Standard
			LEAF			
P. s. TBR	121.2±5.4	130.4±7.8	146.2±2.4	91.32±1.7	103.2±5.9	10.2±0.5[€]
P. s. DC	73.6±2.2	82.5±5.3	107.3±3.8	62.6±2.6	85.3±1.2	16.9±0.9[€]
P. s. NPS	54.7±1.3	63.7±2.2	95.3±2.3	52.4±1.9	54.5±1.4	15.8±0.7[€]
			FLOWER			
P. s. TBR	76.5±0.4				62.7±0.3	
P. s. DC	50.4±0.7				45.4±0.1	
P. s. NPS	31.9±0.8				26.7±0.6	

P.s. TBR, *Pseudomonas syringae* pv. tabaci TBR2004
P.s. DC, *Pseudomonas syringae* pv. tomato DC3000
P.s.NPS, *P. syringae* pv. phaseolicola NPS3121
[€] Ag, Agrigent Plus® used as antibiotic of reference
[***] MIC values are expressed in µg mL^{-1}
[b]SP, spring; SU, summer; FA, Fall; WI, winter.

Table No 4
Antibacterial activity of the majoritarian volatiles of the essential oil from *Z. montagnifolia* on *Pseudomonas syringae* pathovars (MIC±SD).

[***]Bacteria	Camphor	Germacrene D	β-caryophyllene	Limonene
P. s. TBR	289.1±0.5	38.3±2.2	54.2±1.3	284.3±2.4
P. s. DC	321.6±2.2	35.4±4.9	36.5±3.8	299.4±1.5
P. s. NPS	324.6±2.6	66.2±3.6	39.2±3.9	276.7±1.8

P.s. TBR, *Pseudomonas syringae* pv. tabaci TBR2004
P.s. DC, *Pseudomonas syringae* pv. tomato DC3000
P.s.NPS, *P. syringae* pv. phaseolicola NPS3121
[***] MIC values are expressed in µg mL^{-1}

CONCLUSION

The chemical profile of the seasonal essential oils from the aerial parts from *Z. montagnifolia* was described for the first time. Thirty two compounds were differentially detected in the studied years. Camphor and germacrene D were the most abundant volatiles in all the seasons studied. The seasonal essential oils from the aerial parts showed anti-HMG-CoA reductase activity, camphor and borneol were evidently involved in such activity. The essential oils showed a relevant growth inhibitory activity on three *Pseudomonas syringe* strains with special emphasis in the pathovar phaseolicola. Among the studied volatiles, germacrene D and β-caryophyllene were the sesquiterpenes with the strongest antibacterial effect.

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