Emirates Journal of Food and Agriculture. 2017. 29(9): 724-728
doi: 10.9755/ejfa.2017.v29.i9.99
http://www.ejfa.me/

SHORT COMMUNICATION

Chemical profile, nutraceutical and anti-phytobacterial properties of the essential oil from *Dalea foliolosa* (Fabaceae)

Nemesio Villa-Ruano[1]*, Yesenia Pacheco-Hernández[2], Efrain Rubio-Rosas[3], José A. Zarate-Reyes[3], Edmundo Lozoya-Gloria[4], Ramiro Cruz-Duran[5]

[1]Universidad de la Sierra Sur, Guillermo Rojas Mijangos S/N, Ciudad Universitaria CP 70800, Miahuatlán de Porfirio Díaz Oaxaca, México, [2]Centro de Investigación en Biotecnología Aplicada-IPN, Ex-Hacienda San Juan Molino Carretera Estatal Tecuexcomac-Tepetitla Km 1.5, CP 90700, Tlaxcala, México, [3]Centro Universitario de Vinculación y Transferencia de Tecnología, Benemérita Universidad Autónoma de Puebla, CP 72570, Puebla, México, [4]Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato, Km 9.6 Carretera Irapuato-León, CP 36821, Irapuato, Guanajuato, México, [5]Facultad de Ciencias UNAM, Ciudad Universitaria, México DF, Ciudad Universitaria, CP 04510, Del. Coyoacán, México, D.F.

ABSTRACT

The present work describes the chemical composition of the leaf essential oils from the annual plant *Dalea foliolosa,* which was collected in three consecutive years (2014-2016). The amount of monoterpenes (69.5-84.6%) was higher to that of sesquiterpenes (10.3-22.9%) and aliphatic hydrocarbons (1-2.1%). Cryptone (22.3-30.6%) was the most abundant oxygenated monoterpene, followed by linalool (10.4-17.6%), caryophyllene oxide (4.6-15.3%), ascaridole (4.5-7.4%) and β-citronellol (3.8-5.6%). The hydrodistillated extract showed antioxidant activity against DPPH radical (IC_{50}, 45-156 μg mL^{-1}), strong anti-α-glucosidase activity (IC_{50}, 14-47 μg mL^{-1}) and inhibited the growth of *Pseudomonas syringae* pv. tabaci TBR2004 (MIC, 44-105 μg mL^{-1}) and *P. syringae* pv. tomato DC3000 (MIC, 35-155 μg mL^{-1}). The possible use of the essential oil as a novel additive for anti-hyperglycemic supplements and for the biological control of the analyzed *Pseudomonas syringae* varieties, may be contemplated.

Keywords: Antibacterial; Antioxidant; Anti-α-glucosidase *Dalea foliolosa* essential oils.

INTRODUCTION

Essential oils and its endogenous volatiles are gaining attention in the fields of food technology and biological control (Tongnuanchan and Benjakul, 2014). Thus, scientific research performed on aromatic plants, represents a promising alternative for the discovery of novel food additives and antimicrobials. The *Dalea* genus is comprised by approximately 470 species, many of them considered as medicinal and/or aromatic plant sources (Woods and Hughes, 2013). Currently, little is known about the volatile composition and biological activities of many members of the *Dalea* genus. Up to now, only the volatile profiles of *D. coerulia*, *D. formosa* and *D. strobilacea* have been formally published (Arango et al., 1994; Lucero et al., 2005; Benites et al., 2016). *Dalea foliolosa* is an annual plant included in the Electronic Atlas of the Mexican Traditional Medicine because of its anti-inflammatory and hypoglycemic properties (UNAM, 2017). The plant usually grows in the xeric scrublands of several provinces from Mexico including the states of Puebla, Tlaxcala, Morelos, Oaxaca and San Luis Potosí (Villaseñor and Espinosa, 1998). In these geographical regions, the plant is traditionally boiled to prepare aqueous infusions against type 2 diabetes mellitus or is locally administered to treat contusions and abrasions. Considering the unknown chemistry and the possible uses of *D. foliolosa* in food technology and agriculture, this work reports the chemical characterization of the essential oil and the first approaches that demonstrate its potential use in these fields.

***Corresponding author:**
Nemesio Villa-Ruano. Universidad de la Sierra Sur, Guillermo Rojas Mijangos S/N, Ciudad Universitaria CP 70800, Miahuatlán de Porfirio Díaz Oaxaca, México. E-mail: necho82@yahoo.com.mx

Received: 01 March 2017; **Revised:** 20 September 2017; **Accepted:** 25 September 2017; **Published Online:** 05 October 2017

MATERIALS AND METHODS

Plant material

Dalea foliolosa (Aiton) Barrneby (Fabaceae) was collected in Miahuatlán de Porfírio Díaz, Oaxaca, México (16° 34.31' N, 0.96° 58.07' W, 1580 masl) during 2014-2016. The identity of the plant was certified at the Herbarium-FCME-UNAM-Mexico where a reference voucher 150042 was deposited.

Extraction of the essential oil from *D. foliolosa*

Leaves of *D. foliolosa* were collected in February 2014, January 2015 and January 2016. The material was immediately dried at 30°C for 3 days in darkness. Dried leaves (300 g) from five different plant samples (five for each season, n=5) were extracted by hydrodistillation in a Clevenger type apparatus for 3 h in order to obtain five different essential oils. The hydro-distillation process was repeated several times using 30 g of dried material per 100 mL of distillated water to complete 300 g. The hydro-distillated extract was dissolved in *n*-hexane (J.T Baker). Posteriorly, the solvent was removed under N_2 stream and traces of water were dried over anhydrous sodium sulfate. The essential oils were stored at 4 °C in amber glass vials until analyzed. The yields and density of the essential oils were determined as previously described by Villa-Ruano et al. (2015a).

The GC-MS and GC-FID analyses were performed on a Hewlett Packard 6890 II series with a HP-5 capillary column (30m x 0.25 mm, 0.25 μm of 5:95 phenyl-dimethylpolysiloxane). The mobile phase was helium at 1 mL min^{-1} flow rate. The run conditions were the same previously reported by Villa-Ruano et al. (2015b). The GC-MS data were additionally corroborated in a Varian CP3800 gas chromatograph equipped with a generic capillary column Factor Four VF-5 ms (30 m x 0.25 mm I.D, 25 μm of 5:95 phenyl-dimethylpolysiloxane plate) coupled to a Varian quadrupole 320MS model. The volatiles were identified by comparison of their mass spectra (70 eV) and retention indices with those available in the NIST 2.0 Standard Reference Database and with the literature (Adams, 2007). Some of those metabolites were additionally identified by the co-injection of authentic standards. The relative Kovats retention index (RRI) was obtained by running a standard mixture of *n*-alkanes (C_8-C_{20}, C_{21}-C_{40}, Sigma-Aldrich Co.) under the same conditions. The quantification of the components was performed based on their GC-FID peak areas. The results were expressed as means (n=5) plus or minus standard deviation.

Antioxidant and anti-α-glucosidase activities

Both *in vitro* assessments were carried out using the DPPH radical and the α-glucosidase enzyme commercially available from Sigma-Aldrich Co. Dose response curves of 0.01-0.15 mg mL^{-1} were performed in quintuplicate for each essential oil previously emulsified in absolute ethanol following the procedures described by Villa-Ruano et al. (2015b). Ascorbic acid (Sigma-Aldrich Co.) and acarbose (Medi-Mart Labs®) were used as standards of reference for antioxidant and anti- α-glucosidase tests, respectively. The IC_{50} was calculated for each essential oil by linear regression (GraphPad 6.05) and the results were expressed as means (n=5) plus or minus standard deviation (IC_{50}±SD). The data were validated by an ANOVA-Tukey test ($p<0.01$).

Antimicrobial activity

The annual essential oils were tested for its antibacterial activity against the phytopathogenic *Pseudomonas syringae* pv. tabaci TBR2004 and *P. syringae* pv. tomato DC3000 by the broth microdilution method (Sarker et al., 2007) and using the same media and incubation times previously described by Villa-Ruano et al. (2015b). Dose-response curves of 0.005-0.30 mg mL^{-1} were done in with the essential oils (previously emulsified in absolute ethanol) and Agrigent Plus® as a standard of reference. The incubations were performed with $5x10^5$ cfu of each phytopathogenic bacteria in a 96-well plate in quintuplicate at a final volume of 0.3 mL. Changes in the colorimetric reactions were monitored at 545 nm using rezasurin (Sigma-Aldrich Co.) as indicator of cell viability (Sarker et al., 2007). The MIC value was considered as the first concentration able to avoid any absorbance change in the dose response curves. The data were expressed as means (n=5) plus or minus standard deviation (MIC±SD) and validated by an ANOVA-Tukey test ($p<0.01$).

RESULTS AND DISCUSSION

The yellow crystalline essential oil from *D. foliolosa* showed variable yields (2.5 to 3.2% w/w) during the time of study (five samples per year). These yields were higher than those reported for some *Eucalyptus* species and to those of *D. formosa* and *D. strobilacea* (Lucero et al., 2005; Elaissi et al., 2012; Benites et al., 2016). The number of replicates revealed the infra-specific variation of volatiles in *D. folioliosa* and the general changes in the chemical profile during the three consecutive years studied. The chemical composition of the studied essential oils revealed a dominance of monoterpenes over sesquiterpenes and aliphatic hydrocarbons (Table 1). Cryptone (22-30%) was the principal oxygenated monoterpene followed by linalool (10-17%), caryophyllene oxide (4-15%), ascaridole (4-8%) and β-citronellol (3-7%). According to these results, the chemical profile of *D. foliolosa* was quite different to that of *D. coerulea, D. formosa* and *D. strobilacea* (Arango, 1994; Lucero et al., 2005; Benites et al., 2016). However, the total monoterpene content seems similar in these species. It is known that *Eucalyptus* plants biosynthesize high amounts

Table 1: Chemical composition of the essential oils from the leaves of *D. foliolosa*, the results are presented as the mean of five samples (n=5) plus or minus standard deviation

Compound	LRI	RRI	Samples			Identification
			2014 (%)	2015 (%)	2016 (%)	
Nonane	900	900	1.5±0.19	-	0.6±0.17	N, MS, A, S
Sabinene	972	971	0.9±0.14	-	0.7±0.16	N, MS, A
β-Pinene	976	975	2.4±0.29	3.5±0.42	0.5±0.15	N, MS, A, S
p-Cymene	1024	1025	1.9±0.27	0.6±0.11	0.8±0.12	N, MS, A
Limonene	1033	1032	2.6±0.32	2.9±0.41	1.6±0.23	N, MS, A, S
Eucalyptol	1037	1037	0.7±0.21	0.7±0.14	1.3±0.22	N, MS, A, S
β-Phellandrene	1030	1029	0.6±0.14	1.9±0.17	1.6±0.17	N, MS, A
cis-Linalol oxide	1074	1074	7.3±0.56	2.9±0.33	4.8±0.24	N, MS, A
Linalool	1098	1097	17.6±1.51	14.8±0.99	10.4±0.13	N, MS, A, S
Verbenol	1139	1137	1.5±0.24	-	1.1±0.23	N, MS,
Terpinen-4-ol	1182	1181	0.8±0.15	-	0.7±0.13	N, MS, A
Cryptone	1186	1186	25.7±3.11	22.3±1.83	30.6±2.42	N, MS, A
α-Terpineol	1189	1187	0.7±0.13	-	0.5±0.12	N, MS A
cis-Piperitol	1204	1203	-	4.6±0.50	0.9±0.17	N, MS, A
β-Citronellol	1224	1223	5.6±0.84	7.9±0.85	3.8±0.53	N, MS, A
m-Cumenol	1225	1224	1.5±0.18	-	0.8±0.10	N, MS, A
Cumin aldehyde	1235	1234	-	0.6±0.16	0.5±0.16	N, MS, A
Ascaridole	1236	1237	7.4±0.93	8.9±0.67	4.5±0.43	N, MS, A
Piperitone	1258	1257	0.6±0.10	2.6±0.29	0.5±0.11	N, MS, A
Geranial	1269	1269	6.8±0.86	3.5±0.41	3.4±0.43	N, MS, A
Borneol, acetate	1289	1289	-	0.7±0.21	0.5±0.19	N, MS, A
Copaene	1375	1374	-	1.5±0.18	0.9±0.19	N, MS, A
β-Bourbonene	1384	1383	-	0.8±0.17	1.4±0.20	N, MS, A
Tetradecane	1400	1401	0.6±0.12	-	0.4±0.14	N, MS, A
Aromadendrene	1431	1433	-	2.3±0.12	1.5±0.21	N, MS, A
Ledol	1564	1566	-	0.9±0.14	0.6±0.14	N, MS, A
Spathulenol	1576	1576	-	0.8±0.10	0.7±0.19	N, MS, A
Caryophyllene oxide	1581	1582	4.6±0.51	9.3±0.13	15.3±0.54	N, MS, S, A
α-Cadinol	1653	1652	5.7±0.9	1.4±0.21	2.5±0.29	N, MS, A
Monoterpenes			84.6±5.6	78.4±4.6	69.5±3.4	
Sesquiterpenes			10.3±1.4	17.1±1.9	22.9±1.7	
Aliphatic hydrocarbons			2.1±0.9	0	1±0.12	
Total			97.0±4.9	95.4±5.8	93.4±7.4	

LRI, retention index for HP-5 column reported in the library of NIST 2.0 Standard reference database (N) and in accordance with Adams 2007 (A), RRI: Relative retention index for the same column or generic columns under experimental conditions, compounds identified by their mass spectra (MS) and additionally by co-injection of authentic standards (S). (-), undetected

of cryptone (Elaissi et al., 2012). Interestingly, the cryptone content of the essential oils from *D. foliolosa* was more abundant than that reported for eight *Eucalyptus* species (Elaissi et al., 2012). Other remarkable finding of this work was the detection of high amounts of ascaridole. The abundance of this volatile was analogous to that described for the essential oils of *Chenopodium quinoa* and *Chenopodium betrays*, plants that accumulate high endogenous levels of the anthelmintic monoterpene (Dembitsky et al., 2008). The levels of cryptone were higher in the essential oils of 2016, but, the amounts of linalool and caryophyllene oxide decreased and increased, respectively. The levels of ascaridole were similar in 2014 and 2015 with a decrease in 2016. The modifications in the endogenous levels of these volatiles could be associated to the interaction of the wild *D. foliolosa* plants with variable biotic and abiotic factors.

All the assayed essential oils showed an inhibitory activity against the DPPH radical and α-glucosidase enzyme (Table 2). Interestingly, the antioxidant and anti-α-glucosidase activities were maintained during the studied years. According to the IC_{50} values, the hydrodistillated extract from 2016 inhibited both systems more effectively than the rest ($p<0.01$). Despite the standards of reference were more effective than the essential oils samples, these preparations could be alternatively used as natural antioxidant and/or anti-α-glucosidase agents. Therefore, its possible use as food additive should be considered. The observed biological activities may be correlated with the levels of cryptone and caryophyllene oxide in the essential oils from 2016. Further experiments with these compounds are required to evaluate its involvement in the anti-α-glucosidase effect. The antioxidant and anti-α-glucosidase

Table 2: Antioxidant and anti-α-glucosidase activities of the essential oils from *Dalea foliolosa*, the results are presented as the mean of five essential oil assayed (n=5) plus or minus standard deviation

Activity	Samples			** Standard
	2014	2015	2016	
*Antioxidant	156.3±12.9[a]	127.8±15.6[b]	45.7±2.6[c]	2.5±0.03
*Anti-α-glucosidase	133.6±2.3[a]	47.2±5.2[b]	14.4±1.4[c]	1.4±0.05

* IC_{50} values are expressed in μg mL^{-1}, **the standards of reference were ascorbic acid for antioxidant activity and acarbose for anti-α-glucosidase activity, [a,b,c] values with diverse letter indicate statistically significant differences ($p<0.01$)

properties were more potent than those recently reported for the essential oil from *Clinopodium macrostemum* (Villa-Ruano et al., 2015b). Additionally, the essential oils from *D. foliolosa* contained several volatiles coincidently found in the essential oil from *Laurus nobilis*. This essential oil was already tested for their antioxidant activity, but also for their effects on α-glucosidase (Basak and Candan, 2013). The *in vitro* anti-α-glucosidase activity reported in the present study partially supports the traditional use of the plant for the treatment of type 2 diabetes mellitus. Nevertheless, further *in vivo* assays with murine models are required to endorse the anti-hyperglycemic property of *D. foliolosa*.

The annual essential oils exhibited a strong antibacterial activity against *Pseudomonas syringae* pv. tabaci TBR2004 and *P. syringae* pv. tomato DC3000 (Table 3). Despite the significant differences among the MIC values ($p<0.01$), the antibacterial activity was maintained during the time of study. These assayed species are associated to the black spot disease of agronomic crops such as potato, tobacco, tomato and bean. Therefore, the present study could be considered as a preamble for subsequent *in vivo* trials using diseased plants. *P. syringae* pv. tabaci TBR2004 was more sensitive to the essential oils containing high levels of cryptone and caryophyllene oxide, whereas *P. syringae* pv. tomato DC3000 was affected by the essential oils containing high amounts of cryptone and linalool. Interestingly, the chemistry of the studied essential oils revealed other volatiles with cytotoxic activity on *Pseudomonas* species (Vasinauskiene et al., 2006). Therefore, the synergistic activity of the volatiles dissolved in the tested essential oils cannot be discarded. Remarkably, the growth inhibitory properties of the essential oils from *D. foliolosa* were more effective than those previously reported for oils with similar activity on the same bacterial strains (Villa-Ruano et al., 2015a; 2015b). Contrary to the slight antibacterial activity of the essential oil from *D. strobilacea* on Gram positive species, the essential oil from *D. foliolosa* showed a significant effect on Gram negative bacteria (Benites et al., 2016).

Table 3: Antibacterial activity of the essential oils from *Dalea foliolosa*, the results are presented as the mean of five MIC values (n=5) plus or minus standard deviation

Bacteria	Samples			Standard
	2014***	2015***	2016***	
**P. s. TBR*	64.3±7.3[b]	105.2±12.4[a]	44.7±1.3[c]	10.1±0.5[€]
***P. s. DC*	35.6±4.5[c]	98.4±4.9[b]	155.3±24.5[a]	15.9±0.7[€]

**P.s.* TBR, *Pseudomonas syringae* pv. tabaci TBR2004, ***P.s.* DC, *Pseudomonas syringae* pv. tomato DC3000, €Ag, Agrigent plus® used as antibiotic of reference, ***MIC values are expressed in μg mL^{-1}, [a,b,c] values with diverse letter indicate statistically significant differences ($p<0.01$)

CONCLUSIONS

The volatile profile of the Mexican medicinal plant *D. foliolosa* was presented for the first time. Twenty-nine compounds were differentially identified during the time of study. Cryptone, linalool, caryophyllene oxide, ascaridole and β-citronellol were the main bioactive volatiles detected during three consecutive years. The annual essential oils showed significant *in vitro* antioxidant, anti-α-glucosidase and antibacterial capacities at less than 0.2 mg mL^{-1}. Further studies in successive years could reveal changes in the chemical profile and biological activities.

ACKNOWLEDGMENTS

This work was partially supported by a sabbatical research project granted by CONACyT-Mexico (Conv.291122- Solic.457483).

Author contributions

N.V.R and Y.P.H, collected the plant material, performed the extraction of the essential oil and performed the GC/GC-MS, antioxidant and anti-α-glucosidase experiments. E.R.R and J.A.Z.R contributed in GC-MS analyses. E.L.G. contributed in the revision of the data as well as in the interpretation of the results and in the language corrections of the MS. R.C.D. performed the certification of the plant. All the authors participated in the structuration of the article.

REFERENCES

Adams, R. P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. 4th ed. Allured Publishing Co., Carol Stream, I.L, USA.

Arango, B. A. I., G. J. González, Z. J. E. Luque and B. Moreno. 1994. Potential insecticidal activity of sesquiterpenes present in *Dalea coerulea* (L.f.) Schinz. et Thellung. Agron. Colomb. 11: 164-174.

Basak, S. S. and F. Candan. 2013. Effect of *Laurus nobilis* L. essential oil and its main components on α-glucosidase and reactive oxygen species scavenging activity. Iran. J. Pharm. Res. 12: 367-379.

Benites, J., C. Moiteiro, A. C. Figueiredo, P. Rijo, P. Buc-Calderon, F. Bravo, S. Gajardo, I. Sánchez and M. Ganoza. 2016. Chemical composition and antimicrobial activity of essential oil of Peruvian *Dalea strobilacea* Barneby. Bol. Latinoam. Caribe Plant Med. Aromat. 15: 429-435.

Dembitsky, V., I. Shkrob and L. O. Hanus. 2008. Ascaridole and related peroxides from the genus *Chenopodium*. Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech. Repub. 152: 209-215.

Elaissi, A., Z. Rouis, N. B. A. Salem, S. Mabrouk, Y. Salem, K. B. H. Salah, M. Aouni, F. Farhat, R. Chemli, F. Harzallah-Skhiri and M. L. Khouja. 2012. Chemical composition of 8 *Eucalyptus* specie's essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. BMC Complement Altern. Med. 12: 1.

Lucero, M. E., R. E. Stell and R. L. Sedillo. 2005. The composition of *Dalea formosa* determined by steam distillation and solid phase microextraction. J. Essent. Oil Res. 17: 645-647.

Sarker, S. D., L. Nahar and Y. Kumarasamy. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 42: 321-324.

Tongnuanchan, P. and S. Benjakul. 2014. Essential oils: Extraction, bioactivities, and their uses for food preservation. J. Food Sci. 79: R1231-R1249.

UNAM. 2017. Electronic Atlas of the Mexican Traditional Medicine. Available from: http://www.medicinatradicionalmexicana.unam.mx/monografia.php?l=3&t=&id=7714. [Last cited on 2017 Feb 27].

Vasinauskiene, M., J. Radusiene, I. Zitikaite and E. Surviliene. 2006. Antibacterial activities of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agron. Res. 4: 437-440.

Villa-Ruano, N., Y. Pacheco-Hernández, E. Rubio-Rosas, E. Lozoya-Gloria, C. Mosso-González, L. G. Ramón-Canul and R. Cruz-Durán. 2015a. Essential oil composition and biological/pharmacological properties of *Salmea scandens* (L.) DC. Food Control. 57: 177-184.

Villa-Ruano, N., Y. Pacheco-Hernández, R. Cruz-Durán and E. Lozoya-Gloria. 2015b. Volatiles and seasonal variation of the essential oil composition from the leaves of *Clinopodium macrostemum* var. *Laevigatum* and its biological activities. Ind. Crop Prod. 77: 741-747.

Villaseñor, R. J. L. and F. J. G. Espinosa. 1998. Catálogo de malezas de México. Universidad Nacional Autónoma de México. 1st ed. Consejo Nacional Consultivo Fitosanitario, Fondo de Cultura Económica, México.

Woods, M. and W. S. Hughes. 2013. The genus *Dalea* (*Fabaceae*) in Alabama. Phytoneuron. 30: 1-12.