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Chemical composition and antimicrobial activity of essential oil of peruvian *Dalea strobilacea* Barneby

[Composición química y actividad antimicrobiana del aceite esencial de la planta peruana *Dalea strobilaceae* Barneby]

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Abstract: The composition of the essential oil obtained by hydrodistillation from *Dalea strobilacea* Barneby (Fabaceae) aerial parts was examined by GC and GC/MS. β-Phellandrene (44%) together with α-pinene (18%) were the main essential oil components. Antimicrobial activity of the essential oil was evaluated against eight bacterial strains. A moderate growth inhibition of *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* was shown by the essential oil.

Keywords: *Dalea strobilacea*, essential oil, chemical composition, antimicrobial activity, Fabaceae.

Resumen: La composición del aceite esencial de *Dalea strobilacea* Barneby (Fabaceae) obtenido por hidrodestilación de las partes aereas fue examinada por CG y CG/EM. β-felandreno (44%) junto con α-pineno (18%) fueron los principales componentes del aceite esencial. La actividad antimicrobiana del aceite esencial fue evaluada contra ocho cepas bacterianas. El aceite esencial inhibió moderadamente el crecimiento de *Klebsiella pneumoniae*, *Staphylococcus aureus* y *Enterococcus faecalis*.

Palabras clave: *Dalea strobilacea*, aceite esencial, composición química, actividad antimicrobiana, Fabaceae.

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INTRODUCTION

Essential oils are lipophilic molecules responsible for flavours and fragrances. Their isolated constituents are widely used as antioxidants and antimicrobial agents as well as for both prevention and treatment of different human diseases (Burt 2004; Pichersky *et al*., 2006). Essential oils are now attracting increasing interest in the scientific community and there is much research being performed on their pharmacological activities. A particular interest has been focused on their antimicrobial and antioxidant properties, which are important for food preservation and the treatment of diseases provoked by bacterial and viral infections, inflammations, cancers and cardiovascular diseases, including atherosclerosis and thrombosis (Sonboli *et al*., 2005).

The genus *Dalea*, Fabaceae family, is composed by around 10,000 species and is highly diversified in the northern Peruvian Andes, some of these species have been used as medicines (Baldeón *et al*., 2006). As part of our research program focused on the evaluation of the popular use of medicinal plants of the Chilean and Peruvian Andean highlands, the biological activities of several plants has been investigated (Rojo *et al*., 2006; Rojo *et al*., 2009; Benites *et al*., 2009; Benites *et al*., 2011).

In this work we selected *Dalea strobilaceae* Barneby, because its use by residents for reducing gastrointestinal smooth muscle spasm and digestive disorders (stomach distress and indigestion). Its infusion is highly prized as breakfast tea for its mild flavour that replaces the lemon verbena. This species is known by the vernacular name "hierba de chil" (Sánchez, 2011).

Regarding the chemical composition of essential oils from genus *Dalea*, data are scarce in the literature. Moreover, no reports have been published about the chemical composition of the essential oil from *Dalea strobilacea* Barneby collected in the region of Cajamarca, Perú. Therefore, we decided to carry out a study to determine *Dalea strobilacea* essential oil composition, and to explore its potential biological activity, specifically antimicrobial activity.

MATERIALS AND METHODS

Plant material

Dalea strobilacea Barneby plants were collected in March 2011 in the Community of Chugur at 2648 m above sea level, in the province of San Marcos, Department of Cajamarca, Perú (Figure 1). Once collected, the specimen was identified by Prof. Isidoro Sánchez from the Herbarium Caxamarcense of the Cajamarca University. A voucher sample under accession No. 12602 was deposited in this herbarium.

Figure 1
Picture of *Dalea strobilacea*, from Cajamarca, Perú. (Taken by Mayar Ganoza, on March 2014).

Essential oil isolation

Fresh aerial parts (40 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil yield was calculated based on a moisture-free basis as 0.90% (w/w). The obtained oil was dried over anhydrous sodium sulfate and after filtration, stored at +4° C until tested and analyzed.

Gas chromatography Analysis

The essential oil was analyzed on a Perkin Elmer Clarus 400 gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (polydimethylsiloxane, 30 m x 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column [(50% phenyl)-methylpolysiloxane, 30 m x 0.25 mm i.d., film thickness 0.15 µm; J & W Scientific Inc.]. Oven temperature was programmed, 45-175° C, at 3° C/min, subsequently at 15° C/min up to 300° C, and then held isothermal for 10 min; injector and detector temperatures, 280° C and 300° C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm/s. The samples were injected using split sampling

technique, ratio 1:50. The volume of injection was 0.1 µL of a pentane-oil solution (1:1). The percentage composition of the oil was computed by the normalization method from the GC peak areas, calculated as mean values of two injections from oil, without using correction factors.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the essential oil was conducted on a Perkin Elmer Clarus 600 gas chromatograph, equipped with DB-1 fused-silica column (30 m x 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific, Inc.), and interfaced with a Perkin-Elmer Clarus 600T mass spectrometer (software version 4.1, Perkin Elmer, Shelton, CT, USA). Injector and oven temperatures were as above; transfer line temperature, 280° C; ion source temperature, 220° C; carrier gas, helium, adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy, 70 eV; scan range, 40-300 m/z; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C_9-C_{21} *n*-alkane indices and GC-MS spectra from a home made library, constructed based on the analyses of reference oils, laboratory-synthesized components and commercial available standards.

Representative Microbial Groups by ATCC Reference Strains

Bacterial strains: the microorganisms used were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 23357), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 106996), *Enterococcus hirae* (ATCC 10541) and *Bacillus subtilis* (ATCC 6633).

Antimicrobial Activity

The minimum inhibitory concentrations (MICs) by the essential oil, was determined by means of the two-fold serial broth microdilution assay (Wayne, 2008). The essential oil, dissolved in dimethylsulphoxide (DMSO), was diluted at concentrations ranging from 500 to 0.488 µg/mL, with Mueller-Hinton broth medium. The antimicrobial activity of the solvent was also evaluated. Vancomycin and norfloxacin were used as positive controls. The MIC values (µg/mL) were taken as the lowest concentration of the essential oil inhibiting bacterial growth, after 24h of incubation at 37º C. The microorganism growth was measured with an Absorbance Microplate Reader set at 620 nm (Termo scientific Multiskan FC). Assays were carried out in triplicate for each tested microorganism.

RESULTS AND DISCUSSION

In total, fifty-one compounds were identified in *Dalea strobilacea* essential oil, accounting for 97% of the total composition (Table 1). Monoterpene hydrocarbons were the major constituents (82%), while the oxygen-containing monoterpenes, were present in concentrations of 5%. The sesquiterpene hydrocarbons were prevalent (7%) as compared to oxygen-containing sesquiterpenes (3%). In addition, phenylpropanoids were present in lower concentrations in oil (0.1%).

Comparing the present data (Table 1) with those previously reported in literature, the studied essential oils of genus *Dalea* displayed different chemical profiles, although oxygenated terpenes have been reported as the main constituents of the essential oils of several species as for example *Dalea lumholtzit* which contained up to 78% of oxygenated terpenes (McCaughey and Buehrer 1961). However, in *Dalea strobilacea* essential oil (Table 1), the main compounds were: β-phellandrene (44%), α-pinene (18%), limonene (3%) and δ-cadinene (3%).

Essential oils have been traditionally investigated for some standard biological activities like antimicrobial, fungicide and antioxidant (Abed 2007). Moreover, the antimicrobial properties are likely due to the presence of active monoterpene constituents (Gao *et al.*, 2011). Since monoterpenes hydrocarbons are the major constituents (82%), we were interested to determine whether the essential oil from *Dalea strobilacea* has a potential antimicrobial activity. To this end, three Gram-negative (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) and five Gram-positive (*S. aureus*, *S. epidermidis*, *E. faecalis*, *E. hirae* and *B. subtilis* strains) were selected to cover a broad spectrum antimicrobial activity.

Table 2 shows the activity of *D. strobilacea* essential oil against both Gram-negative and Gram-positive bacterial strains. The inhibitory effect on bacteria growth was compared to that showed by Vancomycin, a glycopeptide antibiotic currently used in the prophylaxis and treatment of infections caused by Gram-positive bacteria. Regarding Gram-negative bacteria the effect caused by the oil was quite different: no activity against *E. coli* (as it was the case by using Vancomycin); a rather modest inhibitory effect against *P. aeruginosa* as compared to

Table 1
Percentage composition of the essential oil isolated from *Dalea strobilacea* Barneby, collected in Cajamarca, Perú.

Compound	RI[a]	Relative content (%)	Identification method
Tricyclene	921	t	RI, MS
α-Thujene	924	1.0	RI, MS
α-Pinene	930	17.7	RI, MS
Camphene	938	0.2	RI, MS
Sabinene	958	1.5	RI, MS
β-Pinene	963	4.5	RI, MS
Myrcene	975	5.1	RI, MS
α-Phellandrene	995	2.1	RI, MS
α-Terpinene	1002	0.2	RI, MS
p-Cymene	1003	1.2	RI, MS
1,8-Cineole	1005	t	RI, MS
β-Phellandrene	1005	43.5	RI, MS
Limonene	1009	3.0	RI, MS
cis-β-Ocimene	1017	0.1	RI, MS
trans-β-Ocimene	1027	1.4	RI, MS
γ-Terpinene	1035	0.5	RI, MS
trans-Sabinene hydrate	1037	t	RI, MS
Terpinolene	1064	0.2	RI, MS
Linalool	1074	1.8	RI, MS
trans-*p*-2-Menthen-1-ol	1099	0.2	RI, MS
cis-*p*-2-Menthen-1-ol	1110	0.2	RI, MS
Citronellal	1121	0.2	RI, MS
Cryptone	1143	0.2	MS
Terpinen-4-ol	1148	1.0	RI, MS
α-Terpineol	1159	0.2	RI, MS
Estragole	1163	0.1	RI, MS
cis-Piperitol	1182	t	MS
trans-Piperitol	1189	0.1	MS
Citronellol	1207	0.8	RI, MS
Eugenol	1327	t	RI, MS
α-Cubebene	1345	0.2	RI, MS
α-Copaene	1375	0.5	RI, MS
β-Cubebene	1385	0.1	RI, MS
β-Elemene	1388	0.1	RI, MS
β-Caryophyllene	1414	0.4	RI, MS
β-Copaene	1426	0.1	RI, MS
trans-Cadina-1(6),4-diene	1469	0.2	RI, MS
γ-Muurolene	1469	0.2	RI, MS
Germacrene-D	1474	0.7	RI, MS
Cubebol	1486	0.9	MS
α-Muurolene	1494	0.5	RI, MS
γ-Cadinene	1500	1.4	RI, MS

***trans*-Calamenene**	**1505**	**0.3**	**RI, MS**
δ-Cadinene	**1505**	**2.6**	**RI, MS**
Spathulenol	**1551**	**0.3**	**RI, MS**
β-Caryophyllene oxide	**1561**	**0.1**	**RI, MS**
Gleenol	**1569**	**0.2**	**MS**
Ledol	**1580**	**0.3**	**RI, MS**
T-Cadinol	**1616**	**0.2**	**RI, MS**
δ-Cadinol	**1618**	**0.9**	**RI, MS**
α-Cadinol	**1626**	**0.2**	**RI, MS**

[a] RI - Retention index as determined on the DB-1 column using the homologous series of n-alkanes (C_9-C_{21}); t - trace (< 0.05)

Table 2
Minimum inhibitory concentration (MIC) of essential oil of aerial parts from *Dalea strobilacea* Barneby.

Microorganism	Gram-/+	MIC[a]	
		Essential oil (□g/mL)	Standard antibiotic (□g/mL)
Escherichia coli	G-	n.d [b]	Va[c] n.d [b]
Klebsiella pneumoniae	G-	59.5	Va[c] 15.4
Pseudomonas aeruginosa	G-	>125	Nor[d] <0.48
Staphylococcus aureus	G+	62.5	Va[c] <0.48
Staphylococcus epidermidis	G+	>125	Va[c] 1.95
Enterococcus faecalis	G+	7.81	Va[c] >125
Enterococcus hirae	G+	>125	Va[c] 0.98
Bacillus subtilis	G+	>125	Va[c] <0.49

[a] MIC = Minimum inhibitory concentration
[b] nd = antibacterial activity not detected
[c] Standard antibiotic: Va = Vancomycin
[d] Standard antibiotic: Nor = Norfloxacin

Norfloxacin, and a quite similar effect against *K. pneumoniae* when compared to Vancomycin. With regard to Gram-positive microorganisms, the oil was more active than Vancomycin against *E. faecalis*. However, the inhibition of bacterial growth caused by the oil was largely lower than Vancomycin against the three other bacteria strains. The results of antimicrobial activity showed that the essential oil had varying degrees of growth inhibition against the microorganisms tested. The differential sensitivity of microorganisms to both Vancomycin and oil has may be explained in terms of variability in the penetration rate through cell wall and cell membrane structures. Indeed, the higher resistance among Gram-negative bacteria can be ascribed to their cell wall structure and outer membrane arrangement as well as on the type of essential oil (Gao *et al*., 2011; Cox *et al*., 2000). The mechanism of action by terpenes is not fully understood. It is thought to involve membrane disruption by the lipophilic compounds (Cox *et al*., 2000; Cowan 1999) but the inhibition of a specific respiratory enzyme or metabolic event cannot be excluded. Therefore, the antibacterial activity may be related to the chemical proportion of the main compounds, β-phellandrene (44%), α-pinene (18%), as well as the minor components present in the essential oil. Since essential oils are quite complex mixtures the contribution of each constituents as well as a potential synergistic effect between in the observed antimicrobial effect them is still unclear. Additional experiments conducted with individual compounds are required to answer this point. Indeed, as nicely discussed by Jiang *et al*., the complex chemical composition makes it often difficult to explain the biological activities shown by essential oils (Jiang *et al*., 2011).

CONCLUSIONS

Based on our results we can conclude that β-phellandrene together with α-pinene were the main constituents of the essential oils from *Dalea strobilacea* Barneby. The essential oil was active against a *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis*.

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El Dr. Isidoro Manuel Sánchez Vega nació en el distrito de Moche, Región La Libertad, Perú, realizó sus estudios en la Universidad Nacional de Trujillo donde obtuvo el grado de Bachiller en Ciencias Biológicas, Título Profesional de Biólogo y Doctor en Ciencias Biológicas, asimismo Maestro en Ciencias por el Colegio de Posgraduados, Chapingo – México. Fue Socio Honorario de la Sociedad Peruana de Botánica así como de la Botanical Society of America, USA. Profesor Principal de la Facultad de Ciencias Agrícolas y Forestales de la Universidad Nacional de Cajamarca, Profesor Honorario y Doctor Honoris Causa de la Universidad Privada Antonio Guillermo Urrelo (UPAGU). Investigador Asociado en el Field Museum of Natural History, Chicago, USA y del Ohio State University Herbarium, USA. Fundador y Director del Herbario de la Universidad Nacional de Cajamarca (CPUN) y fundador del Herbario de la Universidad Privada de Piura. Organizó y dirigió diversas expediciones científicas con interés en la Botánica del nororiente peruano, siendo conocido como el "Padre de la Jalca".

Durante sus más de cincuenta años de fructífera labor científica, se destacan más de una veintena de artículos científicos en publicaciones nacionales e internacionales, trece libros de su especialidad, más de quince mil colecciones botánicas, contribuyó con treintaidós especies nuevas para la ciencia, nueve de ellas dedicadas a él, a mencionar *Ascidiogyne Sanchez- vegae* Cabrera, *Dalea isidorii* Barneby, *Verbesina sanchezii* Sagastegui, *Caxamarca sanchezii* M.O. Dillon & Sagastegui, *Siphocampylus sanchezii* Lammers, *Jaltomata sanchez-vegae* S. Leiva & Mione, *Ribes sanchezii* Wigend, *Passiflora sanchezii* Weigend, *Solanum sanchez-vegae* S. Knapp.

El Dr. Isidoro siempre se caracterizó por su sencillez y humildad dignas de un Maestro Universitario, fue un ciudadano muy respetado y considerado en la ciudad de Cajamarca, por su don de gente y honestidad; iniciamos hace cuatro años en la UPAGU el proyecto Mapa de Vegetación de Cajamarca. Potencialidad de la vegetación para el uso de plantas medicinales, que él mismo dirigió conjuntamente con el Dr. Antonio Galán de Mera de la Universidad CEU San Pablo, Madrid, España, el cual culminaremos y publicaremos honrando su memoria.

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