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Phytochemical evaluation and antibacterial activity of *Espeletia schultzii* (Asteraceae) inflorescences

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Abstract

The increasing interest for the medicinal properties in plants is what induced the present work and was selected for the ethnobotanical information the species *Espeletia schultzii* Wedd. (Frailejón de Octubre), of the family Asteraceae, to accomplish an in vitro evaluation of its antibiotic and phototoxic activities. This species is used for the treatment of asthma in folk medicine in the Venezuelan Andes. A phytochemical evaluation was carried out which confirmed that the inflorescences extract contains kaurenic acids, it was isolated and identified benzophenone and two flavonoids, identified as quercetin and its 3-0-galactoside. These are the first flavonoids found in this species, and this is the first report of benzophenone in genus *Espeletia*. Three fractions obtained of the ethyl acetate extract, as well as the aqueous residual extract, were selected to accomplish an *in vitro* evaluation of their antibiotic and phototoxic activities. These activities were tested employing bacteria of clinical origin. Only one of the ethyl acetate fractions and the aqueous residual were found active against at least one bacterial strain. As far as we know, this study constitutes the first report on evaluation of the antibacterial activity of *E. schultzii* Wedd.

Key words: Antibacterial activity, Asteraceae, *Espeletia shultzii*, flavonoids, treatment asthma.

Evaluación fitoquímica y actividad antibacteriana de las inflorescencias de *Espeletia schultzii* (Asteraceae)

Resumen

El presente trabajo es el producto del actual y creciente interés por las plantas medicinales, y basándose en la exploración etnobotánica se escogió la especie *Espeletia schultzii* Wedd. (Frailejón de Octubre), Asteraceae, para evaluar *in vitro* su actividad antibacteriana y fototóxica. Esta especie es utilizada entre la población andina como un remedio para el asma. La evaluación fitoquímica realizada a las inflorescencias confirmó la presencia de ácidos kaurénicos y se reporta el aislamiento e identificación de ácidos kaurénicos, benzofenona y dos flavonoides, identificados como la quercetina y su 3-O-galactósido. Estos son los primeros flavonoides aislados en esta especie, y éste es el primer reporte de benzofenona en el género *Espeletia*. Tres fracciones obtenidas del extracto de acetato de etilo, así como, el extracto acuoso residual, fueron

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seleccionados para evaluar sus actividades antibacteriana y fototóxica. Estas actividades se ensayaron con varias bacterias de origen clínico. Sólo una fracción del extracto de acetato de etilo y el extracto acuoso residual mostraron actividad antibacteriana contra alguna cepa bacteriana. Este trabajo constituye el primer reporte de estudios realizados sobre la actividad antibacteriana de la especie *Espeletia schultzii* Wedd.

Palabras clave: Actividad antibacteriana; asma; Asteraceae; *Espeletia shultzii;* flavonoides.

Introduction

On the basis of ethnobotanical information, the species *Espeletia schultzii* Wedd. (Frailejón de Octubre), of the family Asteraceae, was selected for an *in vitro* evaluation of its antibiotic and phototoxic activities. This species is widely distributed in the Páramo, Mérida State, Venezuela, about 2.900-4.300 m (1), and is used as a remedy for the treatment of asthma in the folk medicine of the Venezuelan Andes. Chemical studies carried out on this plant reported the isolation and identification of kaurenic acids, hydroxyacetophenone derivatives (2, 3, 4), and qualitative detection of glycosylated flavonoids (5). In the present study the inflorescences of this plant were evaluated phytochemically and biologically.

Material and Methods

Plant collection

The *Espeletia schultzii* Wedd. (Asteraceae) species was collected in Venezuela, Mérida State, Municipio Justo Briceño, Páramo of Piedras Blancas, about 13 Km from the Pico El Aguila, toward Piñango way (Contreras 157). The specimen was taxonomically classified by Forest technician Giuseppe Adamo, Centro Jardín Botánico, Facultad de Ciencias, Universidad de Los Andes, and a voucher specimen, # 2527, is kept in the MERC Herbarium of this Faculty.

Extraction and isolation

About 900 g of the inflorescences dried and ground were submitted to extraction with organic solvents of increasing polarity and water. The extracts were analyzed by bidimensional paper chromatography with TBA (terbuthanol: acetic acid: water 3:1:1) and acetic acid (15 %) as eluents. The chromatograms were visualized with NH_3. The dichloromethane extract (40.4 g) was fractionated in a silica gel column, 28 fractions were collected and similar fractions were combined from which three compounds were isolated. The ethyl acetate extract (50.3 g) was submitted to various sephadex LH-20 and polyamide column chromatography, thirty-four fractions were collected and similar fractions were combined from which two compounds were isolated and purified using sephadex LH-20 columns.

Spectroscopic data

Compound 1. Benzophenone:

White powder. IR ν_{max} cm^{-1}: 3088, 1653, 1448, 1416, 919, 814, 775. ^{1}HNMR (200 MHz): δ 7.76 (2H, dd, H-3), 7.64 (1H, tt, H-5), 7.52 (2H, td, H-4). ^{13}CNMR (50 MHz): δ 198.60 (C-1), 138.95 (C-2), 130.88 (C-3), 129.45 (C-4), 133.70 (C-5).

Compound 2. Grandifloric acid ent-15-β-hydroxy-16-en-kaur-19-oic acid:

White powder. IR ν_{max} cm^{-1}: 3500, 3303, 2983, 1692. MS m/e: 318 (M^+). ^{1}HNMR (200 MHz, C_5D_5N): δ 5.52 and 5.23 (1H, s, H-17), 4.18 (1H, s, H-15), 2.75 (1H, m, H-13), 1.20 (3H, s, H-18), 1.37 (3H, s, H-20). ^{13}CNMR (50 MHz): δ 41.2 (C-1), 19.9 (C-2), 38.8 (C-3), 44.0 (C-4), 57.3 (C-5), 22.1 (C-6), 36.7 (C-7), 48.4 (C-8), 54.1 (C-9), 40.3 (C-10), 18.8 (C-11), 33.1 (C-12), 42.9 (C-13),

36.4 (C-14), 82.7 (C-15), 161.0 (C-16), 107.7 (C-17), 29.3 (C-18), 180.1 (C-19), 16.3 (C-20).

Compound 3. Grandiflorenic acid [ent-kaur-9 (11),16-dien-19-oic acid]:

White powder. IR ν_{max} cm^{-1}: 3374, 3100, 2976, 1692, 950-876. ^{1}HNMR (200 MHz): δ 1.02 and 1.24 (3H, s, H-18 and H-20), 2.77 (1H, m, H-13), 5.24 (1H, t br, H-11), 4.91 and 4.79 (1H, s, H-17). ^{13}CNMR (50 MHz): δ 40.85 (C-1), 20.19 (C-2), 38.32 (C-3), 44.79 (C-4), 46.70 (C-5), 18.52 (C-6), 29.76 (C-7), 42.31 (C-8), 156.02 (C-9), 38.85 (C-10), 114.05 (C-11), 37.95 (C-12), 41.30 (C-13), 45.03 (C-14), 50.39 (C-15), 158.50 (C-16), 105.44 (C-17), 28.25 (C-18), 184.10 (C-19), 23.59 (C-20).

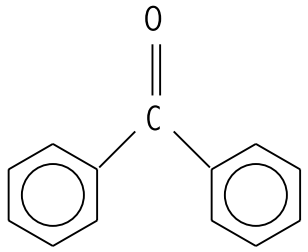
Compound 4. Quercetin:

Yellow powder. UV λ_{max} nm: (MeOH) 372, 300 sh, 257; (NaOMe) 412, 328, 260; ($AlCl_3$) 443, 273; ($AlCl_3$/HCl) 430, 355 sh 308 sh, 270; (NaOAc) 400, 328, 275; (NaOAc/H_3BO_3) 390, 293, 260. ^{1}HNMR (200 MHz, DMSO-d_6): δ 12.5 (1H, s, OH in C-5), 10.8 (1H, s, OH in C-3), 9.6 (1H, s, OH in C-7), 9.3 (1H, s, OH in C-3´/C-4´), 7.7 (1H, d, J= 2.2 Hz, H-2´), 7.5 (1H, d d, J= 2.2, 9 Hz, H-6´), 6.9 (1H, d, J= 9, H-5´), 6.4 (1H, d, J=2.2, H-8), 6.2 (1H, d, J= 2.3, H-6). ^{13}CNMR (50 MHz, DMSO-d_6): δ 146.68 (C-2), 135.60 (C-3), 175.71 (C-4), 160.69 (C-5), 98.05 (C-6), 163.75 (C-7), 93.23 (C-8), 156.01 (C-9), 102.89 (C-10), 121.83 (C-1´), 114.93 (C-2´), 144.93 (C-3´), 147.57 (C-4´), 115.47 (C-5´), 119.85 (C-6´).

Compound 5. Quercetin 3-O-galactoside:

UV λ_{max} nm: (MeOH) 358, 298 sh, 270 sh, 257; (NaOMe) 405, 325 sh, 272; ($AlCl_3$) 434, 335 sh, 300, 272; ($AlCl_3$/HCl) 402, 360 sh, 303 sh, 272; (NaOAc) 390, 320 sh, 270; (NaOAc/H_3BO_3) 373, 295sh, 260. ^{13}CNMR (50 MHz, DMSO-d_6): δ 177.4 (C-4), 164.03 (C-7), 161.1 (C-5), 156.2 (C-2, C-9), 148.3 (C-4´), 144.7 (C-3´), 133.4 (C-3), 121.86 (C-6´), 120.97 (C-1´), 115.82 (C-5´), 115.07 (C-2´), 103.8 (C-10), 98.5 (C-6), 93.4 (C-8), 101.7 (C-1´´), 75.7 (C-5´´), 73.07 (C-3´´), 71.09 (C-2´´), 67.8 (C-4´´), 60.0 (C-6´´).

1



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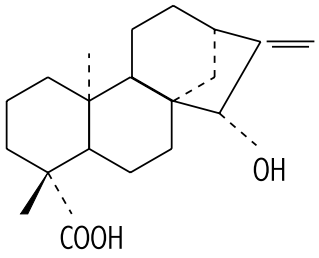

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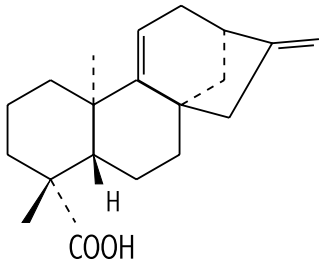

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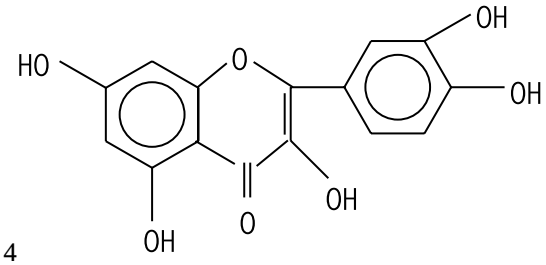

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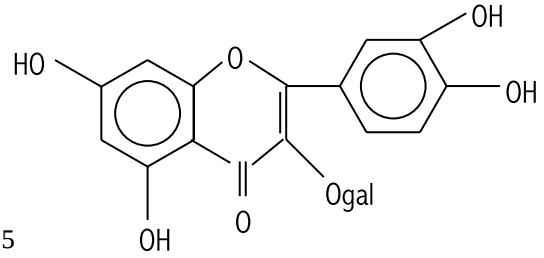

Antibacterial Activity

Three fractions obtained of the ethyl acetate extract, as well as the aqueous residual extract, were selected for an *in vitro* evaluation of the antibacterial activity using the disc-diffuse method (6), and the phototoxicity was performed by the Meckes method (7). These activities were tested employing bacteria of clinical origin, Gram (+) and Gram (-).

Paper discs impregnated with 25 µl of the extract (corresponding to 40 mg/mL), as well as discs impregnated with the solvents used to prepare each solution were placed on sterile Müller-Hinton agar plates, which were inoculated with test bacteria, *Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa*. The plates were pre-incubated for 18 h at 4°C to allow diffusion, and then were kept 24 h in the incubator at 37°C. The sizes of the inhibition zones were measured. To test for light-activated antibacterial activity, one replicate was exposed to UV-A light (200 W, λ = 320-400 nm, from Hanovia lamps) for 2, 4, 6, 8 h, before they were placed on the nutrient agar inoculated with test bacteria, then submitted to the procedure mentioned before. All assays were performed in duplicate.

Results

The bidimensional paper chromatography of the organic extracts obtained from the inflorescences showed that flavonoids of different polarities were present. The dichloromethane and ethyl acetate extract were fractionated with organic solvents and water followed by chromatographic separation and the chemical structures of the compounds obtained were determined. Three compounds were isolated from the dichloromethane extract and identified as benzophenone (1) (40.2 mg), grandifloric acid ent-15-b-hydroxy-16-en-kaur-19-oic acid (2) (80.5 mg), and grandiflorenic acid [ent-kaur-9 (11),16-dien-19-oic acid] (3) (100.3 mg). The structures of the compounds were established by the spectral methods: UV, ^{1}H NMR, ^{13}C NMR, IR, and TLC was compared with an authentic sample. All spectral data were compared with those reported in the literature (8, 9). Two flavonols were obtained from the ethyl acetate extract. The analysis of the UV spectra in MeOH and displacement reactants, carried out on the isolated flavonols, indicated that these had the same pattern of substitution in the positions 5, 7, 3' and 4', and that the substitution in position 3 was different. Comparison of their spectroscopic data UV, ^{1}H NMR, ^{13}C NMR, IR and MS with those reported in the literature (10, 11) and TLC with an authentic sample allowed the identification as quercetin (4) (25.2 mg) and its 3-0-galactoside (5) (18.3 mg).

One of the fractions of the ethyl acetate extract and the aqueous residual extract showed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus,* respectively (Tables 1 and 2).

The exposure to UV-A light had no effect on the activities of the extracts.

Discussion

Espeletia schultzii Wedd. is used for the treatment of asthma, which suggested that some biological activity could be present in this plant. The present experimental study revealed that some extract obtained of this plant showed antibacterial activity against *B. cereus* and *S. aureus in vitro.* This result could be attributed to some components with biological activity present in this plant. The flavonoids have antibacterial properties and it could be that the observed antibacterial activity is related to the presence of flavonoids in the plant. The antibacterial activity of the 3-O-galactoside has been reported in the literature (12). Several investigations carried out on Quercetin report its antiviral properties (13). The genus *Espeletia* is characterizated by the ocurrence of kaurenic ac-

Table 1
Antibacterial and phototoxic activities of one of the ethyl acetate fractions of the inflorescences from *Espeletia schultzii* Wedd

Microorganism	Zone of Inhibition (mm) Irradiation Time (h)				
	0 h	2 h	4 h	6 h	8 h
E. coli	—	—	—	—	—
P. aeruginosa	—	—	—	—	—
S. typhi	—	—	—	—	—
S. aureus	—	—	—	—	—
B. cereus	14	12	14	12	14

— No Zone of Inhibition was observed.

Table 2
Antibacterial and phototoxic activities of the aqueous extract of the inflorescences from *Espeletia schultzii* Wedd

Microorganism	Zone of Inhibition (mm) Irradiation Time (h)				
	0 h	2 h	4 h	6 h	8 h
E. coli	—	—	—	—	—
P. aeruginosa	—	—	—	—	—
S. typhi	—	—	—	—	—
S. aureus	15	14	12	—	—
B. cereus	—	—	—	—	—

— No Zone of Inhibition was observed.

ids. Grandiflorenic acid [ent-kaur-9 (11)-16--en-19-oic acid], which is common in species of *Espeletia* (14), and has been previously isolated from *E. schultzii* (2, 3, 4), is also present in *Montana tomentosa* "Zoapatle" a plant used in Mexican folk medicine, which is reported as an anticonceptive (15).

The evidence of antibacterial activity and the presence of compounds with known biological activity in the studied plant indicate that *E. schultzii* could be a therapeutic source in the treatment of some infectious diseases.

The flavonoids quercetin and its 3-0-galactoside are the first flavonoids found in this species. This is the first report of benzophenone in genus *Espeletia*.

As far as we know, this study constitutes the first report on evaluation of the antibacterial and phototoxic activities of *Espeletia schultzii* Wedd.

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