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Antioxidant Activity of Diterpenes and Polyphenols from *Ophryosporus heptanthus*

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The antioxidant activity of 14 compounds (**1**−**14**) isolated from the ether and butanolic extracts of the aerial parts of *Ophryosporus heptanthus* has been assayed using a β-carotene bleaching method and the DPPH technique. Compounds **1** and **13** showed the most potent antioxidant activity. Their structures have been established by spectroscopic techniques (mainly NMR). Compounds **7** and **12** are new natural products, and their structures have been confirmed by chemical synthesis.

KEYWORDS: Diterpenes; polyphenols; antioxidant activity; *Ophryosporus heptanthus*

INTRODUCTION

Today, numerous diseases are associated with the formation of reactive oxygen and the induction of lipid peroxidation. Reactive oxygen species constitute a mechanism of tissue injury and are significant in processes of inflammation and aging, and in cardiovascular and neurovegetative disorders, among others. The discovery and development of antioxidant compounds is an effective method for the prevention and treatment of these conditions. Antioxidants are also of interest as industrial additives. Essentially, they are used to preserve foods, plastics, rubber, cosmetics, and oils. Following the screening of different extracts of South American plants in a search for antioxidant activity, *Ophryosporus heptanthus* (*1*), a plant found from southeastern Perú to the central region of Bolivia, has been selected. The genus *Ophryosporus* (Fam. *Asteraceae*, tribe *Eupatorieae*) comprise some 40 species growing in South America. The chemistry of several of them has been studied; their common metabolites include acetophenones, chromones, flavones, and flavanones (*2*−*4*) together with *ent*-labdane-type diterpenes (*5*−*8*). One of them, *Ophryosporus axilliflorus*, has shown anti-inflammatory properties (*9*). Earlier works on *O. heptanthus* describe the isolation of different *ent*-labdanes diterpenoids, tremetone, and chromene derivatives and flavonoids (*6*), and study the essential oil from its aerial parts—the major component being santolinatriene (*10*). In the present study, we have isolated and identified 14 compounds (**1**−**14**) from different extracts of *O. heptanthus* aerial parts. Two of these compounds (**7** and **12**) were new natural products. The remaining ones were identified in previous studies (*5*, *6*, *11*−*13*, *15*, *16*). The antioxidant activity of the extracts studied and the isolated compounds was examined.

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MATERIALS AND METHODS

Chemical Analysis. Optical rotations were measured on a 141 Perkin-Elmer polarimeter. IR spectra were recorded on a Mattson Satellite FTIR spectrometer. High-resolution MS were determined on an Autospec-Q VG-Analytical (FISONS) mass spectrometer. NMR spectra were recorded on Bruker AMX 300 spectrometer (δ values given in ppm relative to internal TMS and J values in Hz). Column chromatography (50 cm long × 1 cm inside diameter, 1 m long × 3 cm inside diameter, and 1 m × 5 cm inside diameter) was carried out using silica gel SDS 60 (35−70 μm), eluting with mixtures of hexane/*t*-BuOMe, *t*-BuOMe/EtOAc, and EtOAc/MeOH of increasing polarity. Analytical TLC was performed on layers of silica gel Merck 60G 0.25 mm thick, using a 7% phosphomolybdic acid solution (EtOH) to visualize the spots.

All solvents used were purchased from Scharlab, S. L. (Barcelona, Spain). Chemicals were products of Fluka or Aldrich (Sigma-Aldrich Química, S. A. Madrid, Spain), except 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP), β-carotene, and butylated hydroxyanisole (BHA), which were purchased from Wako Chemicals (Richmond, VA), BHD Chemicals, Ltd. (Poole, U.K.), and E. Merck (Darmstadt, Germany), respectively.

Plant Material. *O. heptanthus* (Schultz-Bip. Ex Wedd.) King & Rob. was collected in Carrasco (Cochabamba, Bolivia) in June 2000. A voucher specimen (MM 1821) was identified and deposited at the herbarium of the National Flora Reserve "Martín Cárdenas" in Cochabamba.

Extraction, Isolation, and Identification of Compounds. Dried and finely powdered aerial parts of *O. heptanthus* (2 kg) were macerated with *t*-BuOMe (10 L) for 5 days at room temperature and subsequently with MeOH (10 L) for 45 days, obtaining 166 g (8.3% with respect to the weight of aerial parts) of *t*-BuOMe extract (extract A) and 225 g (11.3% with respect to the weight of aerial parts) of MeOH extract. The MeOH extract was evaporated at reduced pressure and partitioned between *t*-BuOMe−MeOH−H_2O (10:2:8) (2 L), and the hydroalcoholic phase was partitioned with EtOAc (500 mL × 3) and *n*-BuOH (500 mL × 3), yielding 71.7 g (3.6% with respect to the weight of aerial parts) of *t*-BuOMe extract (extract B), 10.5 g (0.5% with respect to the weight of aerial parts) of EtOAc extract (extract C), and 31.2 g

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(1.6% with respect to the weight of aerial parts) of *n*-BuOH extract (extract D). A 25 g portion of the *t*-BuOMe extract was subjected to column chromatography over silica gel (230 g), which stepwise-eluted with hexane/*t*-BuOMe in a ratio of 100:0 (1000 mL), 99:1 (900 mL), 95:5 (1500 mL), 90:10 (700 mL), 85:15 (750 mL), 80:20 (2850 mL), 70:30 (850 mL), 50:50 (1150 mL), and 0:100 (2100 mL), and with *t*-BuOMe/EtOAc in a ratio of 90:10 (300 mL) (50 mL per fraction). Eleven main fractions were collected (F1−F11). The least polar fraction (F1, 1732 mg) was mainly constituted by esters of fatty acids. F2 (625 mg, hexane/*t*-BuOMe 85:15) was rechromatographed to isolate 176 mg of precocene II (**1**) and 216 mg of 3,5-bis(3-methyl-2-butenyl)-4-hydroxyacetofenone (**2**). The four following fractions were eluted with hexane/*t*-BuOMe 80:20. F3 (600 mg) was rechromatographed to obtain 57 mg of **1** and 200 mg of 5,6-dimethoxytremetone (**3**). F4 (225 mg) and F5 (620 mg) were not studied. F6 (32 mg) contained 32 mg of 15-acetoxy-3β-hydroxy-*ent*-labda-7,13*E*-diene (**4**). F7 (500 mg, hexane/*t*-BuOMe 70:30) was rechromatographed to yield 18 mg of 4,5-dimethoxysalicylaldehyde (**5**) and 320 mg of a mixture of sakuranetin (**6**) and 15-acetoxy-2α-hydroxy-*ent*-labda-8(17),13*E*-diene (**7**). F8 (1423 mg, hexane/*t*-BuOMe 50:50) consisted of 1423 mg of 15-acetoxy-2β-hydroxy-*ent*-labda-7,13*E*-diene (**8**). The last fractions were eluted with *t*-BuOMe. F9 was composed of 2520 mg of 15-acetoxy-2β,3β-dihydroxy-*ent*-labda-7,13*E*-diene (**9**). F10 (210 mg) was constituted of a mixture of **9**, 15,2α-dihydroxy-*ent*-labda-8(17),13*E*-diene (**10**), and 15,2β-dihydroxy-*ent*-labda-7,13*E*-diene (**11**). F11 (180 mg) was rechromatographed to isolate 4 mg of **8**, 20 mg of **9**, 40 mg of **11**, and 164 mg of 15-acetoxy-2β,3β,7β-trihydroxy-*ent*-labda-7,13*E*-diene (**12**).

The composition of the extract B was similar to that of the extract A (seen by ^{1}H NMR and TLC).

The extract C was subjected to column chromatography over silica gel (180 g), which stepwise-eluted with hexane/*t*-BuOMe in a ratio of 50:50 (1050 mL) and 0:100 (1500 mL); with *t*-BuOMe/EtOAc in a ratio of 95:5 (500 mL), 90:10 (700 mL), 70:30 (700 mL), 30:70 (900 mL), and 0:100 (600 mL); and with EtOAc/MeOH in a ratio of 90:10 (900 mL) and 70:30 (1000 mL) (50 mL per fraction). Three main fractions were collected (P1−P3). P1 (500 mg, hexane/*t*-BuOMe 50:50) was not studied. P2 (1750 mg, *t*-BuOMe/EtOAc 95:5) was chromatographed repeatedly to afford 620 mg of 3,5-di-*O*-caffeoylquinic acid (**13**). P3 (1240 mg, EtOAc/MeOH 70:30) was rechromatographed to isolate 195 mg of acacetin-7-*O*-α-L-rhamnopyronosyl (1‴→6″)-β-D-glucopyranoside (**14**).

Compounds 1, 4, 6, 8, 9, and 11. They have been identified by spectroscopic data. These data were consistent with those previously reported (*6*).

Compound 2. It has been identified by spectroscopic data. These data were consistent with those previously reported (*11*).

Compound 3. It has been identified by spectroscopic data. These data were consistent with those previously reported (*12*).

Compound 5. It has been identified by spectroscopic data. These data were consistent with those previously reported (*13*).

Compound 7. Colorless oil. $[\alpha]^{20}_D$ + 8.6° (c 1, $CHCl_3$); IR (film) ν_{max} 3299, 3080, 2955, 2933, 2868, 1733, 1670, 1647, 1456, 1419, 1232, 1026, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (6H, s, H-18, H-20), 0.90 (3H, s, H-19), 1.05 (1H, m, H-5), 1.21 (1H, m, H-3a), 1.36 (1H, dt, J = 4.0, 13.5 Hz, H-6α), 1.42 (1H, m, H-1a), 1.46 (1H, m, H-9), 1.54 (2H, m, H-3b, H-11a), 1.61 (3H, bs, H-16), 1.65 (3H, m, H-1b, H-6β, H-11b), 1.77 (1H, m, H-12a), 1.88 (1H, m, H-7α), 1.96 (3H, s, $COCH_3$), 2.12 (1H, m, H-12b), 2.30 (1H, ddd, J = 2.5, 4.1, 12.7 Hz, H-7β), 4.08 (1H, quint, J = 3.7 Hz, H-2β), 4.44 (1H, bs, H-17a), 4.49 (2H, d, J = 7.1 Hz, H-15), 4.77 (1H, d, J = 1.3 Hz, H-17b), 5.24 (1H, m, H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.9 (s, C-8), 142.9 (s, C-13), 118.2 (d, C-14), 107.1 (t, C-17), 67.9 (d, C-2), 61.4 (t, C-15), 56.9 (d, C-9), 53.3 (d, C-5), 46.7 (t, C-3), 45.9 (t, C-1), 39.7 (s, C-10), 38.6 (t, C-12), 38.2 (t, C-7), 33.4 (q, C-18), 33.1 (s, C-4), 24.7 (q, C-19), 24.6 (t, C-6), 22.3 (t, C-11), 21.1 (q, CO*C*H$_3$), 17.4 (q, C-20), 16.6 (q, C-16); FAB HRMS (*m*/*z*), [M$^+$ + Na] calcd for $C_{22}H_{36}O_3Na$, 371.256215; found 371.256199.

Compound 10. It has been identified by spectroscopic data. These data were consistent with those previously reported (*5*).

Compound 12. Colorless oil. $[\alpha]^{20}_D$ − 7.7° (c 1, $CHCl_3$); IR (film) ν_{max} 3440, 3089, 2958, 2905, 2862, 1735, 1672, 1653, 1457, 1420, 1236, 1114, 1024, 859 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.64 (3H, s, H-20), 0.77 (1H, m, H-11), 0.78 (3H, s, H-19), 0.95 (3H, m, H-18), 1.39 (1H, m, H-1), 1.49 (1H, m, H-6), 1.65 (3H, m, H-1, H-6, H-11), 1.63 (3H, bs, H-16), 1.83 (1H, m, H-12), 1.93 (1H, dd, J = 2.7, 13.3 Hz, H-5), 2.04 (1H, m, H-12), 2.00 (3H, s, $COCH_3$), 2.11 (1H, t, J = 11.0 Hz, H-9), 3.39 (1H, d, J = 2.5 Hz, H-3α), 3.93 (1H, m, H-2α), 4.31 (1H, t, J = 2.8 Hz, H-7α), 4.51 (2H, m, H-15a, H-15b), 4.61 (1H, bs, H-17a), 5.02 (1H, bs, H-17b), 5.21 (1H, bt, J = 6.5 Hz, H-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6 (s, *C*OCH_3), 148.9 (s, C-8), 142.2 (s, C-13), 118.9 (d, C-14), 110.6 (t, C-17), 79.1 (d, C-3), 73.8 (d, C-7), 66.9 (d, C-2), 61.6 (t, C-15), 49.4 (d, C-9), 40.5 (s, C-10), 40.3 (d, C-5), 40.3 (t, C-1), 38.2 (s, C-4), 30.3 (t, C-6), 28.6 (q, C-18), 21.6 (q, C-19), 21.2 (q, CO*C*H$_3$), 20.9 (t, C-11), 16.5 (q, C-16), 14.5 (q, C-20); FAB HRMS (*m*/*z*), [M$^+$ + Na] calcd for $C_{22}H_{36}O_5Na$, 403.246044; found 403.245761.

Compound 13. It has been identified by spectroscopic data. These data were consistent with those previously reported (*15*).

Compound 14. It has been identified by spectroscopic data. These data were consistent with those previously reported (*16*).

Oxidation of 8 with PDC: Preparation of 15. Pyridinium dichromate (PDC) (1.23 g, 3.24 mmol) was added to a solution of **8** (556 mg, 1.6 mmol) in dry *N*,*N*-dimethylformamide (DMF) (4 mL) under an argon atmosphere at 0 °C, and the mixture was stirred at room temperature for 9 h. The solution was diluted with H_2O (250 mL) and extracted with *t*-BuOMe (100 mL × 3). The organic layer was washed with brine (100 mL × 3) and then dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (H/E, 60:40) to give **15** (334 mg, 60%) as colorless oil. $[\alpha]^{20}_D$ + 1.5° (c 1, $CHCl_3$); IR (film) ν_{max} 2962, 2905, 2854, 1738, 1712, 1666, 1441, 1367, 1286, 1232, 1117, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (3H, s, H-20), 0.87 (3H, s, H-19), 1.01 (3H, s, H-18), 1.36 (2H, m, H-11), 1.67 (3H, bs, H-16), 1.69 (3H, bs, H-17), 1.74 (1H, dd, J = 4.9, 11.8 Hz, H-5), 1.92 (3H, m, H-6α, H-9, H-12a), 2.02 (3H, s, $COCH_3$), 2.09 (3H, m, H-1β, H-3α, H-6β), 2.23 (1H, m, H-12b), 2.35 (1H, d, J = 12.4 Hz, H-3β), 2.42 (1H, dd, J = 1.7, 12.6 Hz, H-1α), 4.54 (2H, d, J = 7 Hz, H-15), 5.30 (1H, bt, J = 7.0 Hz, H-14), 5.41 (1H, bs, H-7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 211.6 (s, C-2), 171.0 (s, *C*OCH_3), 141.9 (s, C-13), 134.9 (s, C-8), 122.1 (d, C-7), 119.0 (d, C-14), 61.2 (t, C-15), 56.4 (t, C-3), 54.1 (d, C-9), 53.8 (t, C-1), 49.8 (d, C-5), 43.0 (s, C-10), 41.5 (t, C-12), 39.2 (s, C-4), 32.5 (q, C-18), 25.3 (t, C-11), 24.0 (t, C-6), 22.5 (q, C-19), 21.9 (q, C-17), 21.0 (q, CO*C*H$_3$), 16.5 (q, C-16), 14.4 (q, C-20); FAB HRMS (*m*/*z*), [M$^+$ + Na] calcd for $C_{22}H_{34}O_3Na$, 369.240565; found 369.240882.

Rearrangement of 9: Preparation of 15. Triphenylphosphine (Ph_3P) (1.45 g, 5.5 mmol) and diethyl azodicarboxylate (DEAD) (974 mg, 5.6 mmol) were added to a solution of **9** (200 mg, 0.55 mmol) in toluene (53 mL) under an argon atmosphere at room temperature, and the mixture was refluxed for 24 h. The solvent was removed in vacuo to give a crude residue, which was purified by silica gel CC (H/E, 70:30) yielding 23 mg of a (1:1) mixture of **15** and **18** (*14*), and 101.3 mg of **15**.

Reduction of 15 with Sodium Borohydride ($NaBH_4$): Preparation of 16. $NaBH_4$ (18 mg, 0.47 mmol) was added to a solution of **15** (130 mg, 0.37 mmol) in MeOH (3 mL). The reaction mixture was stirried at room temperature for 1 h. The solvent was removed, and the crude residue was dissolved in *t*-BuOMe (50 mL) and washed with brine (30 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness to afford 119 mg (91%) of **16**. Colorless oil; $[\alpha]^{20}_D$ + 6.9° (c 1, $CHCl_3$); IR (film) ν_{max} 3447, 3018, 2957, 2907, 2867, 1739, 1718, 1670, 1457, 1364, 1232, 1050, 1024, 773 cm-^{1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (3H, s, H-18), 1.01 (3H, s, H-20), 1.27 (3H, s, H-19), 1.28 (3H, m, H-1β, H-5, H-6α), 1.45 (1H, m, H-3α), 1.60 (3H, m, H-3β, H-6β, H-9), 1.70 (3H, bs, H-17), 1.73 (3H, bs, H-16), 2.00 (4H, m, H-1α, H-11a, H-11b, H-12a), 2.06 (3H, s, $COCH_3$), 2.26 (1H, ddd, J = 4.7, 11.5, 14.8 Hz, H-12b), 4.21 (1H, quint, J = 3.8 Hz, H-2), 4.59 (2H, d, J = 7.1 Hz, H-15), 5.37 (1H, tq, J = 1.2, 7.1 Hz, H-14), 5.43 (1H, bs, H-7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.1 (s, *C*OCH_3), 142.6 (s, C-13), 134.9 (s, C-8), 122.6 (d, C-7), 118.6 (d, C-14), 68.2 (d, C-2), 61.4 (t, C-15), 54.9 (d, C-9), 49.4 (d, C-5), 47.2 (t, C-3), 44.8 (t, C-1), 41.9 (t, C-12), 36.3 (s, C-10), 33.9 (q, C-18), 32.3 (s, C-4), 25.7 (t, C-6), 24.5 (q, C-19), 23.8 (t, C-11), 22.1 (q,

Table 1. Antioxidant Activity of Extracts and Pure Components of *O. heptanthus*

compound	IC_{50} (μg/mL)[a]	% inhibition[b]
Extract A	–[c]	22.47
Extract C	9.74	41.65
Extract D	17.6	26.66
1	–	45.71
2	–	6.13
3	–	0
4	–	0
5	>100	0.06
6 + 7	>100	0
8	>100	11.05
9	41.91	13.05
10 + 11	>100	19.70
12	–	3.21
13	0.0003	56.90
14	>100	0.99

[a] DPPH technique. [b] β-Carotene bleaching method. The results are expressed as % activity relative to the standard BHA solution. [c] No activity detected. All results are statistically significant at $p < 0.05$. All experiments were carried out in triplicate.

C-17), 21.0 (q, CO*CH*$_3$), 16.6 (q, C-16), 15.9 (q, C-20); FAB HRMS (*m*/*z*), [M^+ + Na] calcd for $C_{22}H_{36}O_3Na$, 371.256215; found 371.256216.

Preparation of 7. Phenyl selenium chloride (PhSeCl) (4.5 mg, 0.002 mmol) was dissolved in dry CH_2Cl_2 (1.5 mL), producing an orange solution. To this solution, **16** (80 mg, 0.23 mmol) was added. The addition of **16** resulted in an immediate color change from orange to pale yellow. *N*-chlorosuccinimide (NCS) (34 mg, 0.25 mmol) was then added to the reaction. After 3 h, the reaction was completed. This solution was concentrated, and *t*-BuOMe (20 mL) was added. The solution was washed with H_2O and brine. The ether layer was dried over anhydrous Na_2SO_4 and concentrated to give a crude residue which was dissolved in tetrahydrofuran (THF) (1 mL). To this solution was added AcOH (1 mL) and dust Zn (105 mg). The reaction mixture was stirred at room temperature for 15 h. The resulting mixture was diluted with *t*-BuOMe (10 mL). The ether layer was decanted from the solid, washed with saturated sodium bicarbonate until CO_2 evolution ceased and brine (30 mL × 3), and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the crude residue after chromatography (H/E, 70:30) yielded **7** (61.6 mg, 77%).

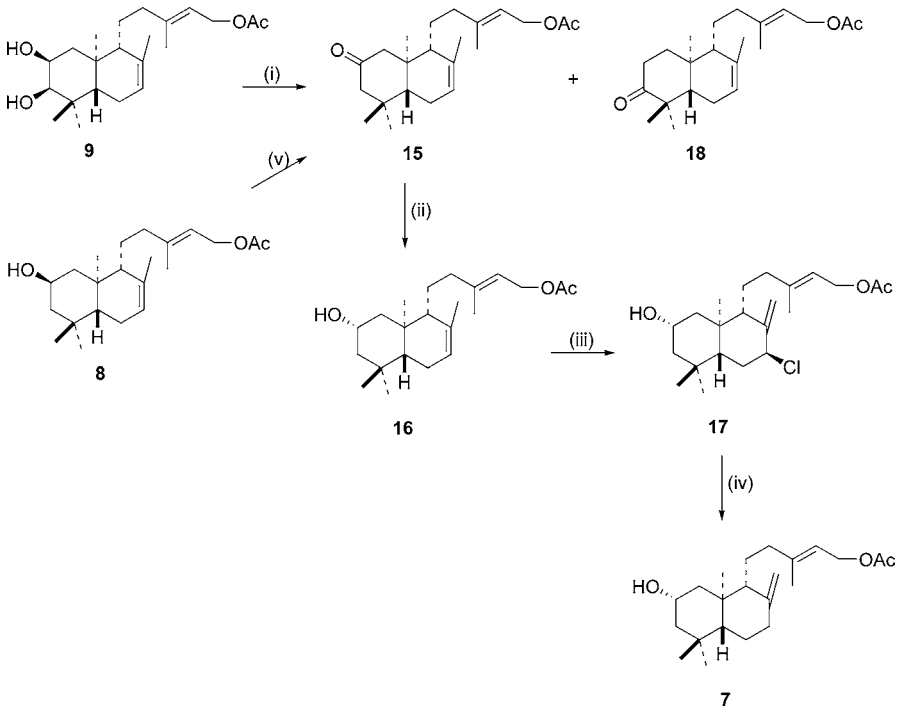
Photooxidation of 9: Preparation of 12. To a solution of **9** (550 mg, 1.51 mmol) in *i*-PrOH (60 mL), Rose Bengal (5 mg) was added, and the solution was exposed to sunshine for 10 h. The solvent was removed, yielding a syrup, which was dissolved in dimethyl sulfide (Me_2S) (20 mL). The solution was kept at room temperature for 12 h. The Me_2S excess was removed in vacuo to give a crude residue, which was suspended in H_2O (50 mL) and extracted with *t*-BuOMe (25 mL × 3). The organic phase was dried over anhydrous Na_2SO_4 and removed to give a crude residue which after chromatography (*t*-BuOMe) yielded **12** (400 mg, 70%).

Antioxidant Activity Tests. *β-Carotene Bleaching Method.* Cell 1 (spectroscopic reference cell, 100% T) contained 2100 μL of a solution of 100 mg of Tween 20 in 50 mL water. All the other cells were filled with 1990 μL of the β-carotene emulsion (*17*). A period of 6 min was needed until thermal equilibrium was attained at 32.0 ± 0.1 °C. ABAP solution [(10 μL), 54.2 mg of ABAP in 500 μL of argon-saturated purified water] was then added to cells 2 and 17 to initiate the linoleic acid oxidation; each cell was stirred for 20 s, and absorbance measurements were taken at 460 nm every 2 min for 10 min. After this time, 100 μL of the BHA solution (24.6 mg of BHA in 100 mL of dimethyl sulfoxide (DMSO)) was added to the cell 2, and the same volumes of the solutions of the materials (24.6 mg of material in 100 mL of DMSO) to be evaluated were added to cells 3−16, respectively. DMSO (101 μL) was added to cell 17 (control, no antioxidant added). Cells 2−17 were shaken in a vortex for 20 s. Readings were automatically taken every 2 min for 90 min.

For each extract or compound, the control and the reference antioxidant (BHA) were evaluated simultaneously in every experiment to obtain good reproducibility. Water used throughout this work was deionized and triple-distilled, with conductivity less than 4 μS/cm; otherwise, the emulsion would not be homogeneous, nor would the kinetic data be reproducible.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Technique. Cell 1 (reference cell, 100% T) contained 2250 μL of the solvent (2:1 MeOH/H_2O). Cell 2 (A_{DPPH}) contained 1500 μL of the DPPH solution (2 mg of DPPH in 100 mL of MeOH) and 700 μL of water. Cell 3 (A_{blank}) contained 1500 μL of solvent and 750 μL of the sample solution (*18*). Cells 4−17 (A_{sample}) contained 1500 μL of the DPPH solution and 750 μL of the

Scheme 1. Synthesis of Compound **7**



Reagents and conditions: (i) DEAD, Ph_3P, toluene, reflux, 24 h. (ii) $NaBH_4$, EtOH, room temperature, 1 h. (iii) PhSeCl, NCS, CH_2Cl_2, room temperature, 3 h. (iv) Zn dust, AcOH/THF (1:1), room temperature, 19 h. (v) PDC, DMF, room temperature, 9 h.

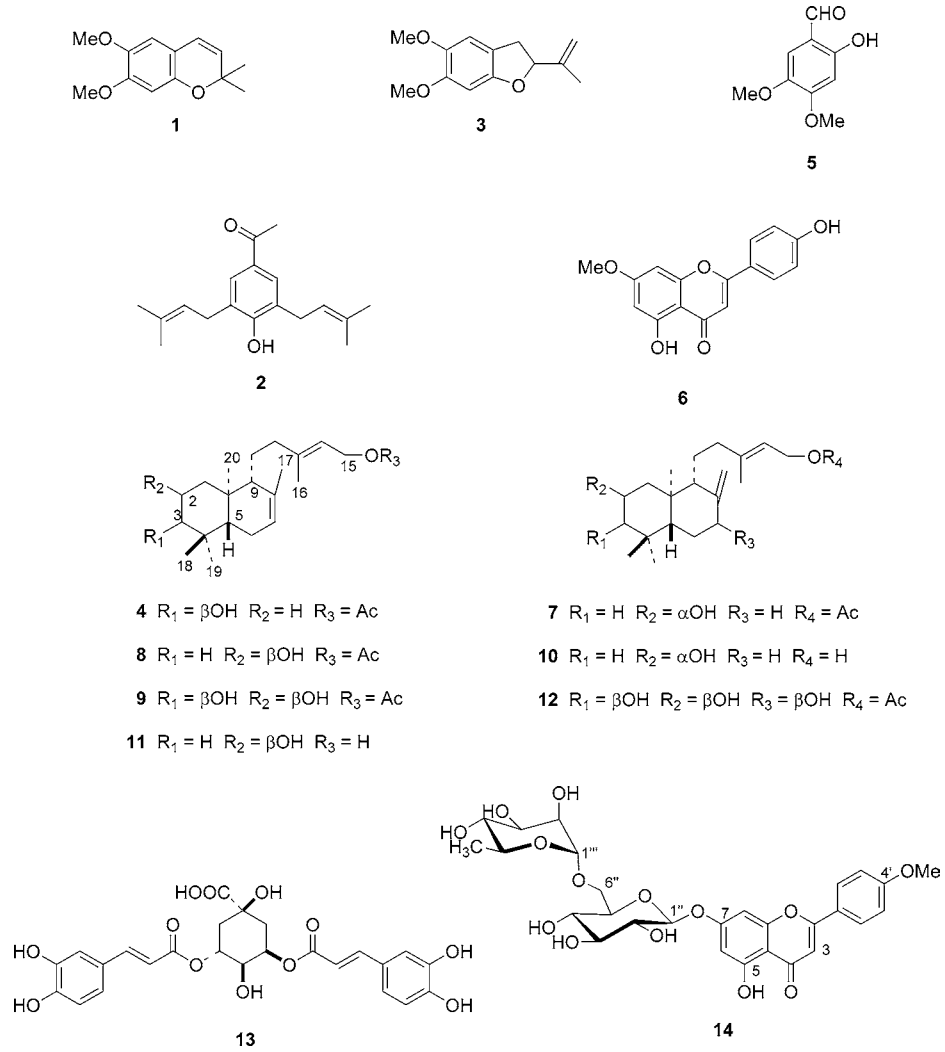


Figure 1. Structures of compounds **1**–**14**.

respective sample solution at room temperature. After 5 min, when the equilibrium was reached, the absorbance was measured at 531 nm (*19*).

The percentage activity for the DPPH technique was calculated according to

$$\% \text{ DPPH} = [1 - (A_{\text{sample}} - A_{\text{blank}})] \times 100/A_{\text{DPPH}}$$

We expressed the DPPH results as the concentration, of extract or compound, necessary to inhit 50% of the free radical oxidation promoting effect of DPPH (EC_{50}) in μg/mL. Concentrations are expressed in μg/mL.

RESULTS AND DISCUSSION

The antioxidant activity of the extracts was examined using the β-carotene bleaching method (*17*), with BHA as standart, and the DPPH technique (*17*). From the results (shown in **Table 1**), extracts A and C were selected for the study. These extracts were subjected to column chromatography over silica gel to afford 14 compounds (**1**−**14**) (**Figure 1**). The structures of compounds **1**−**6**, **8**−**11**, **13**, and **14** were determined by comparison of their spectroscopic data with those reported in the literature (*5*, *6*, *11*−*13*, *15*, *16*). The remaining compounds (**7** and **12**) are new natural products.

Compound **7** presented the molecular formula $C_{22}H_{36}O_3$ deduced from its HRMS. The oxygenated functions can be attributed to an acetate and hydroxyl groups due to the absorption bans in the IR spectrum at 1733 and 3299 cm^{-1}, respectively. The ^{13}C NMR spectrum showed, among others, signals for an acetate group and two double bonds; therefore, the compound **7** contains only two rings. The signals of the ^1H NMR spectrum at δ 0.83 (6H, s), 0.90 (3H, s) and 1.61 (3H, bs) due to four methyl groups, one on a trisubstituted double bond and the remaining ones linked to (at) quaternary carbons, together with those due to an exocyclic double bond at δ 4.44 (1H, bs) and 4.77 (1H, d, J = 1.3 Hz) and a trisubstituted one δ 5.24 (1H, m) are characteristics of a labda-8(17),13-diene skeleton (*20*). The remaining signals in the ^1H NMR and ^{13}C NMR spectra (see Materials and Methods) are in accordance with this labdane skeleton. The hydroxyl group was located at position 2, with axial orientation, based on the pattern of multiplicity and the coupling constant value of H-2 (quintuplet, J = 3.7 Hz). The acetate group was located on C-15, based on the chemical shift and the pattern of multiplicity of H-15 (4.49 ppm, d, J = 7.1 Hz). The stereochemistry *E* for the double bond of the side chain on C-9 was deduced from the chemical shift of the C-16 methyl group (^1H NMR, 1.61 ppm; ^{13}C NMR, 16.6 ppm).

The structure of **7** was confirmed by semisynthesis from **8** or **9** (**Scheme 1**), isolated from *O. heptanthus* (*6*), which also enabled establishing an *ent*-configuration for the labdane skeleton. First, the acetoxy-ketone **15** was obtained by treatment of the diol **9** under Mitsunobu conditions (DEAD/Ph_3P) with

toluene reflux, originating the rearrangement, in neutral medium, of 1,2-diols to carbonyls (*21*). This yielded a 10:1 mixture of the acetoxy-ketones **15** and **18**. The separation of **15** was performed using silica gel column chromatography. The acetoxy-ketone **15** could also be obtained by the oxidation of **8** with PDC in DMF. Stereoselective reduction of the ketone group of **15** with $NaBH_4$ in EtOH, by the more accessible face, led to the acetoxy-alcohol **16**. The isomerization of the double bond 7,8 toward 8(17) position was carried out in two steps, first by allyl chlorination of **16** with PhSeCl/NCS (*22*) followed by chemoselective reduction of the 7β-Cl group of **17** with Zn/AcOH (*23*). Thus, we have obtained the compound **7** in four steps from **8** or **9** with an overall yield of 42.3% or 41.6%, respectively.

The second new natural compound (**12**) presented the molecular formula $C_{22}H_{36}O_5$ (HRMS). Its IR spectrum showed absorption bands of hydroxyl (3440 cm^{-1}) and acetate (1735 cm^{-1}) groups and of double bonds (3089, 1672, and 1653 cm^{-1}). The ^1H NMR and ^{13}C NMR data (see Materials and Methods) indicated a labdane skeleton similar to that of **7**, the main differences being the presence of signals due to two new secondary hydroxyl groups. One of them was located on C-7 (^1H NMR, 4.31 ppm, t; ^{13}C NMR, 73.8 ppm), while the other one on C-3 (^1H NMR, 3.39 ppm, d; ^{13}C NMR, 79.1 ppm). The β-orientation of the hydroxyl group on C-7 was assigned based on the H-7 coupling constant value (J = 2.8 Hz), while the configuration of carbons C-2 and C-3 was determined on the basis of the chemical shift and the coupling constants values measured for H-2 and H-3, which were in agreement with those published for **9** (*6*). The ^{13}C NMR spectrum (see Materials and Methods) is in accordance with the structure of **12**. The structural correlation between **9** and **12** was confirmed by oxidation of **9** by singlet oxygen in *i*-PrOH. This oxidation afforded the corresponding hydroperoxide, which by reduction with dimethyl sulfide yielded **12**.

All the isolated compounds (**1**−**14**) were subjected to the antioxidant activity tests (**Table 1**). 3,5-Di-*O*-caffeoylquinic acid **13** was the most active compound in both assays (β-carotene bleaching method. 56.9% inhibition; and DPPH technique, IC_{50} = 0.0003 μg/mL), presenting a highly interesting antioxidant potency, and was considered responsible for the antioxidant nature of extract C. The use of **13** in cosmetics has been described previously (*24*), as has its antitumoral (*25*), antihypertensive (*26*), collagenase-inhibitory (*27*) activity, and so forth. Extract A showed moderate antioxidant activity in the β-carotene bleaching method (22.47% inhibition), and was inactive in the DPPH technique, and compound **1** was responsible for the activity (β-carotene bleaching method, 45.71% inhibition). Finally, compounds **3**−**12** and **14** showed no activity, or much less than that of compounds **1** and **13**.

These results indicate that this plant can be a source of polar extracts and that the polyphenol 3,5-di-*O*-caffeoylquinic acid (**13**) is responsible for its high activity.

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