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Phytochemical profile of the stems of *Aeonium lindleyi*

***María L. Kennedy*[1, 2]**

[1]*Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Campus UNA, Paraguay.*
[2]*Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, Spain.*

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Abstract: *Aeonium* species are plants rich in phenols and terpenoids and have been widely used in the Canary folk medicine. Following our project concerning bioactive compounds from Macaronesian region, a phytochemical study was carried out to investigate the constituents of the stems of *Aeonium lindleyi* Webb & Berthel.,Crassulaceae. Air dried and chopped stems of this plant were extracted with ethanol in a Soxhlet apparatus and the total extract was chromatographed on silica gel to afford sixteen known compounds including, one sterol, four lupane triterpenes and eleven phenolic compounds. Their structures were elucidated by means of ^{1}H- and ^{13}C-NMR spectroscopic studies and comparison of their spectral data with values in the literature. Both group of isolated metabolites are known by their diverse biological activities and they have been described as antioxidant, cytotoxic, anti-inflammatory. Our results contribute to the knowledge of *A. lindleyi* as a potential source of bioactive compounds.

Introduction

Medicinal plants have been used as sources of medicine in virtually all cultures. In many developing countries, 70 to 95% of the population has used some form of alternative or complementary medicine. In industrialized countries, the use of traditional medicine is equally significant; Canada, France, Germany and Italy for instance, report that between 70 and 90% of their populations have used traditional medicines. Herbal treatments are the most popular form of traditional medicine, and serve to health needs of millions of people in the vast rural areas of developing countries (Robinson & Zhang, 2011).

Crassulaceae is a morphologically diverse and systematically complex Angiosperm family comprising 35 genera and 1500 species. Members of this family are leaf-succulent, usually herbaceous, and often have five-parted, radially symmetrical flowers with two whorls of five stamens each. Reported secondary metabolites from Crassulaceae species are flavonoids, steroids, alkaloids and triterpenoids (Mort et al., 2001).

In Canary Islands the genus *Aeonium* is widely distributed, comprising several species, most of them being used in traditional medicine. In particular, *A. lindleyi* Webb & Berthel., Crassulaceae, "gomereta", an endemic specie of Tenerife and La Gomera Islands, is claimed to be used as anti-inflammatory and anti-pyretic (Pérez de Paz, 1999). Previous researches on *A. lindleyi* have focused on constituents from the leaves and wax (Baker et al., 1962; González et al., 1976, Kennedy et al., 2011).

As part of our project concerning the isolation of biologically active metabolites from plant sources of the Macaronesian region, we have investigated the secondary metabolites of the stems of the medicinal plant *A. lindleyi*. This paper reports on the isolation and structural elucidation of sixteen known compounds from this species.

Materials and Methods

General experimental procedure

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400 MHz and 100 MHz, respectively. $CDCl_3$ (**1-13**) and methanol-d_4 (**14** and **15**) were used as solvents and TMS as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (*J*) in Hz. Silica gel 60 (15-40 μm) for column chromatography, Si gel 60 F_{254} for TLC were purchased from Macherey-Nagel. Purification of the compounds was monitored by TLC and the spots were visualised by UV light and heating silica gel plates sprayed with H_2O-H_2SO_4-AcH (1:4:20).

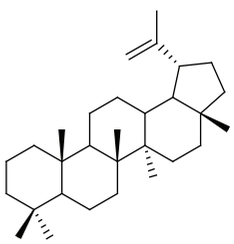
Plant material

Stems of *Aeonium lindleyi* Webb & Berthel., Crassulaceae. locally known as "gomereta", were collected in San Andrés, Tenerife, Canary Islands, Spain, in October 2007 and identified by Leticia Rodríguez-Navarro, Department of Botany, University of La Laguna, Tenerife. A voucher specimen (TFC 49550) is deposited at the herbarium of Department of Botany, University of La Laguna.

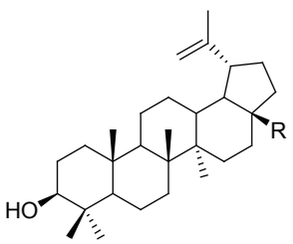
Extraction and isolation of the constituents

Dried-chopped stems (650 g) of *A. lindleyi* were extracted three times, with ethanol 95% in a Soxhlet apparatus. Removal of the solvent under reduced pressure provided 50 g of extract. This extract was chromatographed by dry flash chromatography on a silica gel column, using mixtures of n-hexane-ethyl acetate and ethyl acetate-ethanol of increasing polarity to afford 37 x 200 mL frs., which were reduced to 4 frs. by TLC: A (0-20%, *n*-hexane-ethyl acetate), B (20-40%), C (40-100%), D 100% methanol. Fraction A (2.3 g) was purified by column chromatography on a silica gel column, using mixtures of *n*-hexane-ethyl acetate of increasing polarity (10:0 to 8:2) as eluents to give lupenone (**1**) (Hui & Li, 1976), β-sitosterol (McCarthy et al., 2005) and lupeol (**2**) (Hui & Li, 1976). Fraction B (2.6 g) was purified by column chromatography on a silica gel column, using mixtures of dichloromethane-acetone of increasing polarity (10:0 to 8:2) as eluents to afford betulin (**3**) (Cole et al., 1991), betulinic acid (**4**) (Bringmann et al., 1997), 3,4,5-trimethoxybenzoic acid (**5**) and 4-(4-hydroxyphenyl)-2-butanone (**8**) (known as raspberry ketone). Fraction C (3.3 g) was purified by column chromatography on a silica gel column, using mixtures of dichloromethane-acetone of increasing polarity (9:1 to 7:3) as eluents to afford 1,2,3-benzenetriol (**6**), gallic acid (**7**), 3-hydroxy-4-(4-dihydroxyphenyl)-2-butanone (**9**), 4-(3,4-dihydroxyphenyl)-2-butanone (**10**), 4-(3-hydroxybutyl) phenol (**11**), 4-(2-hydroxyethyl) phenol (**12**) and 4-(2-hydroxyethyl)-1,2-benzenediol (**13**). Fraction D (2.8 of 30.8 g) was purified by column chromatography on a silica gel column, using mixtures of dichloromethane-methanol of increasing polarity (10:0 to 8:2) as eluents to afford 4-[4-(D-glucopyranosyloxy) phenyl]-2-butanone (**14**) (Murakami et al., 1972) and lindleyin (**15**) (González et al., 1976).

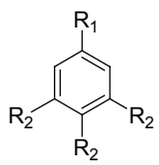
Lupenone (**1**): $[\alpha]^{25}D$ +64.2 (*c* 1.00, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δH 0,80 (*s*, 3H); 0,93 (*s*, 3H); 0,96 (*s*, 3H); 1,03 (*s*, 3H); 1,07 (*s*, 3H); 1,07 (*s*, 6H); 1,66 (*s*, 3H); 2.44 (*m*, 3H); 4.57 (*s*, 1H); 6.69 (*s*, 1H). ^{13}C NMR ($CDCl_3$) δ_C 14,4 (*q*, C-27); 15,8 (*q*, C-25); 15,9 (*q*, C-26); 18,0 (*q*, C-18); 19,3 (*q*, C-30); 19,6 (*t*, C-6); 21,0 (*q*, C-24); 21,5 (*t*, C-11); 25,3 (*t*, C-12); 26,6 (*q*, C-23); 27,0 (*t*, C-15); 29,9 (*t*, C-21); 34,0 (*t*, C-7); 34,1 (*t*, C-2); 35,6 (*t*, C-16); 36,9 (*s*, C-10); 38,0 (*d*, C-13); 39,1 (*t*, C-1); 40,0 (*t*, C-22); 40,9 (*s*, C-8); 42,9 (*s*, C-14); 43,0 (*s*, C-17); 47,3 (*s*, C-4); 47,9 (*d*, C-19); 48,4 (*d*, C-18); 49,8 (*d*, C-9); 54,9 (*d*, C-5); 110,6 (*t*, C-29); 117,0 (*d*, C-3); 150,7 (*s*, C-20). EI-MS *m/z* (rel. int.): 424 (M^+, 34), 410 (17), 381 (20), 368 (9), 313 (20), 205 (56), 191 (56), 109 (100), 95 (96). HREIMS *m/z* 424,3705 (calcd for $C_{30}H_{48}O_1$, 424, 3635).



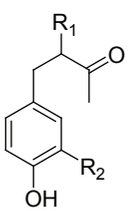
1



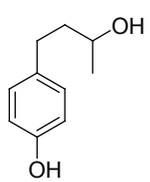
2 R=CH3
3 R=CH_2OH
4 R=CO_2H



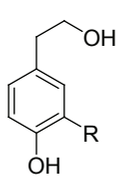
5 R_1=CO_2H; R_2=OMe
6 R_1=H; R_2=OH
7 R_1=CO_2H; R_2=OH



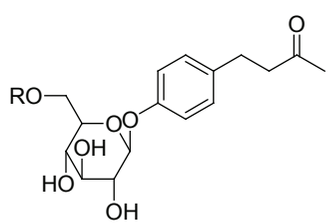
8 R_1=H; R_2=H
9 R_1=OH; R_2=H
10 R_1=H; R_2=OH



11



12 R=H
13 R=OH



14 R=H
15 R=gallic acid

Lupeol (**2**): $[\alpha]^{25}_{D}$ +25.8 (c 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0,77 (*s*, 3H); 0,79 (*s*, 3H); 0,83 (*s*, 3H); 0,95 (*s*, 3H); 0,97 (*s*, 3H); 1,04 (*s*, 3H); 1,68 (*s*, 3H); 2.39 (dt, 1H, *J* =5,6; 10,4 Hz); 3.19 (dd, 1H, *J* =5,5; 10,8 Hz); 4,57 (s, 1H); 4.69 (s, 1H). ^{13}C ($CDCl_3$) δ_C 14,5 (*q*, C-27); 15,3 (*q*, C-24); 15,9 (*q*, C-26); 16,2 (*q*, C-25); 17,9 (*q*, C-28); 18,2 (*t*, C-6); 19,2 (*q*, C-30); 20,9 (*t*, C-11); 25,0 (*t*, C-12); 27,3 (*t*, C-2); 27,3 (*t*, C-15); 27,8 (*q*, C-3); 29.8 (*t*, C-21); 34,1 (*t*, C-7); 35,5 (*t*, C-16); 37,0 (*s*, C-10); 38,1 (*d*, C-13); 38,6 (*t*, C-1); 38,8 (*s*, C-4); 39,8 (*t*, C-22); 40,8 (*s*, C-8); 42,7 (*s*, C-14); 42,8 (*s*, C-17; 47,8 (*d*, C-19); 48,2 (*d*, C-18); 50,2 (*d*, C-9); 55,1 (*d*, C-5); 78,8 (*d*, C-3); 109,0 (*t*, C-29); 150,7 (*s*, C-20). EI-MS *m/z* (rel. int.): 426 (M^+, 46), 408 (33), 365 (25), 339 (12), 297 (17), 218 (100), 189 (94), 109 (92), 95 (100). HREIMS *m/z* 426,3862 (calcd for $C_{30}H_{50}O_1$, 426, 3889).

4-[4-(D-glucopyranosyloxy) phenyl]-2-butanone (**14**): $[\alpha]^{25}_{D}$ -51.8 (c 0.3, MeOH). ^{1}H NMR (400 MHz, methanol-d_4) δ_H 2,10 (*s*, 3H); 2,78 (*s*, 4H); 3,96 (*m*, 4H); 3,68 (*dd*, 1H, *J* =3,9; 11,0 Hz); 3,88 (*d*, 1H, *J* =11,9 Hz); 4,20 (*d*, 1H, *J* =5,4 Hz); 4,85 (*d*, 1H, *J* =10,0 Hz); 7.00 (*d*, 1H, *J* =8,7 Hz); 7,11 (*d*, 2H, *J* =8,7 Hz). ^{13}C (methanol-d_4) δ_C 29.9 (*t*); 30,0 (*q*); 46,0 (*t*); 62,6 (*t*); 71,5 (*d*); 75,0 (*d*); 78,0 (*d*); 78,1 (*d*); 102,6 (*d*); 117,9 (2*xd*); 130,2 (2*xd*); 136,4 (*s*); 156,9 (*s*); 211,8 (*s*). EI-MS *m/z* (rel. int.): 326 (M^+, 5), 307 (34), 285 (23), 273 (6), 176 (14), 153 (100), 135 (69), 106 (16), 88 (13). HREIMS *m/z* 326,3477 (calcd for $C_{16}H_{22}O_7$, 326,3456).

Lindleyin (**15**): $[\alpha]^{25}_{D}$ +14.2 (*c* 0.4, Py). ^{1}H NMR (400 MHz, methanol-d_4) δ_H 2,10 (*s*, 3H); 2,70 (*s*, 4H); 3,45 (*m*, 2H); 3,72 (*d*, 1H, *J* =7,5 Hz); 3,81 (*d*, 1H, *J* =12,4 Hz); 4,40 (*dd*, 1H, *J* =8,0; 11,7 Hz); 4,57 (*dd*, 1H, *J* =1,8; 12,0 Hz); 4,80 (*d*, 1H, *J* =6,6 Hz); 6.87 (*d*, 2H, *J* =8,6 Hz); 6,93 (*d*, 2H, *J* =8,6 Hz); 7.07 (*s*, 2H);. ^{13}C (methanol-d_4) δ_C 29.9 (*t*); 30,0 (*q*); 45,8 (*t*); 65,0 (*t*); 72,11 (*d*); 74,9 (*d*); 75,6 (*d*); 78,1 (*d*); 102,4 (*d*); 110,4 (2*xd*); 117,8 (2*xd*); 121,5 (*s*); 130,2 (2*xd*); 136,3 (*s*); 139,9 (*s*); 146,6 (2*xs*); 157,1 (*s*); 168,2 (*s*); 211,6 (*s*). EI-MS *m/z* (rel. int.): 501 (M^+, 4), 460 (1), 421 (1), 379 (1), 329 (11), 307 (18), 289 (16), 176 (45), 154 (100), 36 (42). HREIMS *m/z* 501,1345 (calcd for $C_{23}H_{26}O_{11}$, 501,1373).

Results and Discussion

As a continuation of our program leaded towards the discovery of bioactive natural products and taking into account that no previous study were reported for the stems of *Aeonium lindleyi* Webb & Berthel.,Crassulaceae. a medicinal plant used as anti-inflammatory and anti-pyretic in the Canary Islands folk medicine we decided to carry out the study of the stems of *A. lindleyi.*

The dried and chopped stems of *A. lindleyi* (650 g) was extracted with ethanol in a Sohxlet apparatus, yielding 50 g of extract after solvent evaporation under reduce pressure. Repeated chromatography of the ethanolic extract on silica gel column afforded sixteen known compounds. Their structures were assigned, based on their spectroscopic data and comparison with those in the literature, as lupenone, β-sitosterol, lupeol, betulin, betulinic acid, 3,4,5-trimethoxybenzoic acid, 1,2,3-benzenetriol, gallic acid, 4-(4-hydroxyphenyl)-2-butanone, 3-hydroxy-4-(4-dihydroxyphenyl)-2-butanone, 4-(3,4-dihydroxyphenyl)-2-butanone, 4-(3-hydroxybutyl) phenol, 4-(2-hydroxyethyl) phenol, 4-(2-hydroxyethyl)-1,2-benzenediol, 4 -[4- (D-glucopyranosyloxy) phenyl]-2-butanone and lindleyin. The isolation of gallic acid and 1,2,3-benzenetriol have been previously reported in Crassulaceae family (Kim et al, 2009) and lindleyin have been isolated previously from *A. lindleyi* (González et al., 1976), while all other phenolic compounds are been described for the first time in the genus *Aeonium.*

Low molecular weight phenolic and pentacyclic triterpenoids are widely distributed throughout the plant kingdom and a variety of pharmacological activity such as antioxidant, gastroprotective, anti-inflammatory, cytotoxic among others have been ascribed to these classes of compounds (Mimica-Dukic & Bozin, 2008; Soldi et al., 2008). Thus, simple phenolic compounds such as gallic acid and their derivatives have showed cytotoxic and anti-proliferative effects (Yanez et al., 2004), gallic acid itself has been identified as the major anti-cancer compound in *Toona sinensis* leaf extracts and it has been shown to have synergistic effect with doxorubicin in suppressing the growth of tumoral cells (Chen et al., 2009), and raspberry ketone demonstrated an anti-obese function and an inhibition of small intestinal absorption of dietary fat (Morimoto et al., 2005). *A. lindleyi* naturally growths in open sunny places, under high intensity light conditions. Under light stress, phenolic content in plants increases and plays an important role in the electrophysiological responses to environmental stresses, due to induce a parallel increase in antioxidant capacity (Reyes & Cisneros-Zeballos, 2003).

Furthermore, one of the most well known member of the pentacyclic triterpene family is betulinic acid which has been shown to exhibit inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic and antioxidant properties (Yogeeswari & Sriram, 2005). In our research group, we have previously reported several lupane triterpenoids from *Maytenus* species as being of interest as inhibitors of nitric oxide and prostaglandin E2, as well as possessing antimicrobial and cytotoxic activities (Núñez et al., 2005: Reyes et al., 2006).

The relevance of our research topic lies in that stems of *A. lindleyi* are sources of lupane triterpenoid, phenolic and phenolic glycosides compounds, all of them

being metabolites of interest because of their wide range of pharmacological properties.

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***Correspondence**

María L. Kennedy
Facultad de Ciencias Químicas, Universidad Nacional de Asunción
Campus UNA, 2169. San Lorenzo, Paraguay
Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna
Avenida Astrofísico Francisco Sánchez 2, 38205 La Laguna, Tenerife, Spain.
lukenrol@qui.una.py
Tel.: + 595 21 585562
Fax: + 595 21 585564