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ORIGINAL ARTICLE

Chemical composition and antioxidant, α-glucosidase inhibitory and antibacterial activities of three *Echeveria* DC. species from Mexico

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Abstract Three *Echeveria* species from Sinaloa, Mexico (*Echeveria craigiana, Echeveria kimnachii* and *Echeveria subrigida*) were analyzed for their content of antioxidant compounds (β-carotene, ascorbic acid, α-tocopherol, total phenolics and flavonoids) and the *in vitro* antioxidant (DPPH, ABTS, ORAC and β-carotene bleaching [β-CBM]), α-glucosidase inhibitory and antibacterial activities. The studied *Echeveria* species showed high α-tocopherol content (2.9–9.0 mg/100 g f.w.) and total phenolics as Gallic Acid Equivalents (GAE) (152.2–400.5 mg GAE/100 g f.w.). Antioxidant activities of the three *Echeveria* methanol extracts (ME) were higher than those of other well-known

Abbreviations: asl, above sea level; ABTS, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; AAPH, 2,2-azobis(2-amidinopropane) dihydrochloride; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; β-CBM, β-carotene bleaching method; CDD, chronic degenerative diseases; CFU, colony forming unit; DPPH, 2,2-diphenyl-1-picrylhydrazyl; f.w., fresh weight basis; GAE, gallic acid equivalents; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; LSD, least significant difference; ME, methanol extract; MBC, minimal bactericide concentration; MIC, minimal inhibitory concentration; pNPG, 4-nitrophenyl-α-D-glucopyranoside; ORAC, oxygen radicals absorbance capacity; PBS, phosphate buffer solution; QE, quercetin equivalents; TFA, trifluoroacetic acid

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plants with this property; the activities of *E. craigiana* (ABTS, 65.91 μmol ET/g f.w.) and *E. subrigida* (β-CBM, 79.3%) were remarkable. The *Echeveria* ME showed stronger α-glucosidase inhibition (IC_{50} 25.21–50.57 μg/mL) than acarbose (IC_{50} 3.59 mg/mL) as well as high antibacterial activity (Minimal Inhibitory Concentrations, MICs ⩽ 1 mg/mL), mainly against Gram positive bacteria. The results showed the three *Echeveria* species had components/biological activities with high potential for food/pharmacological uses and could be exploited by sustainable management schemes.

1. Introduction

All over the world, plants are the main source of food, bioactive compounds (*e.g.* antimicrobial, antioxidant), and those used in traditional medicine are a basic source of new drugs (Conforti et al., 2008; Kennedy, 2012). Nowadays, 25% of the commercial drugs are obtained from a natural product and many of them have been used to treat chronic degenerative diseases (CDD) and infectious diseases (De Luca et al., 2012; Gulati et al., 2012); some of these pathologies are associated with oxidative stress (Ozsoy et al., 2009) and are within the main causes of mortality in the world (*e.g.* CDD 59.7%; diabetes 2.0%; infectious diseases 16.2%) (WHO, 2010). Up to date, treatments against these maladies are not 100% effective and bacterial resistance is aggravating the infectious diseases prognosis. Thus, new drugs are required and plants are still the main reservoir (De Luca et al., 2012).

Recently, the Crassulaceae has emerged as an interesting plant family. Plants of the genera *Sedum* (English common names, *e.g.*, cat's claw and great stonecrop plant), *Kalanchoe* (English common names, *e.g.*, never die and dog's liver plant) and *Rhodiola* (English common name, life plant) show several biological activities (*e.g.* antibacterial, antioxidant, adaptogenic, anti-inflammatory, antidiabetic) (Dimo et al., 2006; Gupta et al., 2010; Szewczyk et al., 2012; Tatsimo et al., 2012). By contrast, the genus *Echeveria* has been poorly studied in spite of its high number of species (> 130) and its common use as a source of ornate plants. This genus is distributed in the American continent and Mexico is considered its center of diversity and endemism with 95% of the registered species (Vázquez-García et al., 2013).

Echeveria plants are traditionally used to treat a number of diseases/symptoms such as diarrhea, oral herpes, inflammation, pain, stomach aches and fever (Instituto Nacional Indigenista, 2009). Only few of these uses are supported with scientific data; it has been demonstrated the contraceptive effect of *Echeveria gibbiflora* (English common name, donkey ear plant) and the antimicrobial activity of *Echeveria leucotricha* (English common name, chenille plant) (Martínez-Ruiz et al., 2012; Reyes et al., 2005).

Our research group is engaged in the study of the agro-industrial potential of wild plants from Sinaloa/Mexico by characterizing their chemicals, nutrients and biological activities. This paper shows the content of antioxidant compounds and the *in vitro* biological activities (antioxidant, α-glucosidase inhibitory and antibacterial) of three *Echeveria* species: *Echeveria kimnachii*, *Echeveria craigiana* and *Echeveria subrigida* (Syn. *Cotyledon subrigida* Robinson & Seaton and *Echeveria angusta* Poelln). These plants are potential sources of active principles for food/pharmaceutical uses.

2. Materials and methods

2.1. Plant material

Echeveria plants were collected from Sinaloa, Mexico; for each species description, we show in parentheses the coordinates, the name of the collector, and the assigned number in the herbarium of the Agronomy Faculty of the Autonomous University of Sinaloa (UAS). *E. craigiana* E. Walther is distributed in the states of Chihuahua, Sonora and Sinaloa; it was collected in "El Zapote" community, Choix, Sinaloa (1050 m above sea level, asl; 26°46′03″N, 108°08′34″O; Vega-Aviña R.; 10816). *E. kimnachii* J. Meyrán & R. Vega is endemic of Sinaloa, specifically of the municipality of Culiacan; it was collected from the South of the "Estancia de los García", Culiacan, Sinaloa (450 m asl; 24°21′45″N, 107°01′05″O; Vega-Aviña R.; 9206). *E. subrigida* (B. L. Rob. & Seaton) Rose is distributed in the Mexico State, Guanajuato, Queretaro and Sinaloa; it was collected near "El Palmito" town, Concordia, Sinaloa (2000 m asl; 23°34′06″N, 105°50′53″O; Vega-Aviña R.; 11742).

Leaves of the same day of collection were used for the analysis in fresh. For the other analyses, leaves were freeze dried (VirTis 25EL, VirTis Co. USA) and milled to obtain a fine flour that passed through a 40 mesh (sample moisture (%) of 95.4 ± 0.0 for *E. craigiana*; 96.5 ± 0.7, *E. kimnachii*; and 93.6 ± 0.1, *E. subrigida*). Dried flours of leaves were stored at −20 °C/in darkness until their use.

2.2. Bacteria

Seven human pathogen bacterial strains were used for the antibacterial assay: two ATCC (*Staphylococcus aureus* 29213 and *Escherichia coli* 25922) (DIFCO Laboratories, MI, USA) and five clinical isolates (*Streptococcus* group A-4, *S. aureus* 3, *E. coli* AO11, *Salmonella enterica* serovar Typhi and *Shigella dysenteriae*) provided by the Laboratory of Bacteriology of the National Institute of Pediatrics, D.F., Mexico.

2.3. Reagents and solvents

The bacteriological culture media were trypticase soy agar and Mueller Hinton broth (Becton Dickinson). The following reagents were purchased from Sigma/Aldrich (St. Louis, MO, USA): β-carotene, ascorbic acid, 4-nitrophenyl-α-D-glucopy-

ranoside (pNPG), α-glucosidase from *Saccharomyces cerevisiae*, 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), trifluoroacetic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, disodium fluorescein, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH). HPLC grade organic reagents were from Baker Inc. (Phillipsburg, NJ, USA). All reagents were of analytical grade.

2.4. Preparation of the methanol extracts

Methanol extracts (ME) of the three *Echeveria* species were obtained by maceration. Flours of *Echeveria* leaves (10 g) were extracted with methanol (1:20 w/v) for three days; the solvent was exchanged every 24 h and the methanol phases were mixed. The solvent was eliminated under vacuum (40 °C) with a rotary evaporator (BÜCHI Labortechnick AG, Switzerland), followed by removal of any residual solvent in a vacuum oven at 40 °C. The obtained ME were stored at −20 °C in darkness until their use.

2.5. Phytochemical characterization

The presence or absence of phytochemicals in the ME of the three *Echeveria* species were determined by tube or thin layer chromatography assays as follows: the Salkowski reaction for terpenes/sterols; the Shinoda test for flavonoids; reaction with 1.0% gelatin solution and quinine sulfate solution with $FeCl_3$ for tannins; lather formation for saponins; yellow fluorescence by reaction with NaOH for coumarins; the Borntträger reaction for free anthracenics; the reagents of Dragendorff and of Mayer and Wagner for alkaloids; and the reagents of Baljet, Raymond–Marthoud, Keller–Killiani, Lieberman–Burchard, and Salkowski for cardiotonics (Harborne, 1973).

2.6. Quantitation of antioxidant components

2.6.1. β-Carotene

β-Carotene was assayed as reported by Valverde-Juárez et al. (2009). *Echeveria* flour (0.3 g de *E. craigiana*/*E. kimnachii* or 0.5 g de *E. subrigida*) was mixed with 2 mL of cold acetone and the mixture was sonicated (Ultrasonic FS30H, Fisher Scientific, USA) for 10 min; the solids were removed by filtration and re-extracted with cold acetone until they were uncolored. The recovered acetone extracts were concentrated under a nitrogen stream, the residue was re-suspended in 1 mL of acetone, passed through syringe filters (17 mm × 0.45 μm, PVDF, Titan), and 100 μL was analyzed with an HPLC-DAD 1100 (Agilent Technologies, USA). The separation was carried out using a YMC carotenoid column (250 mm × 4.6 mm i.d. × 5 μm) (YMC, Wilmington, USA) at 30 °C. The mobile phase contained methanol (A), methyl *t*-butyl ether (B) and water (C). A lineal gradient from 81% A/15% B/4% C to 18.5% A/77.5% C/4% C in 70 min was used. The flow rate was 0.7 mL/min and the eluted components were detected at 447 nm. Quantitation was carried out using a calibration curve prepared with pure β-carotene.

2.6.2. Ascorbic acid

Ascorbic acid was determined as described by Pío-León et al. (2012). Briefly, fresh *Echeveria* leaves were cut and 10 g was extracted with 25 mL cold water; the mixture was homogenized with a waring blender for 20 s, filtered through cheesecloth and the volume of the recovered liquid was measured. Liquid was sequentially passed through a syringe filter (17 mm, 0.45 μm PVDF, Titan) and Sep-Pak C18 cartridge. A 10 μL aliquot of the filtrate was injected to an HPLC-DAD Agilent 1100 and separated by a SPHERECLONE ODS column (250 mm × 4.6 mm i.d. × 5 μm) (Phenomenex, Torrance, CA). The mobile phase used was an aqueous solution of TFA (0.1% v/v) with a flow rate of 0.7 mL/min and detection at 254 nm. Quantitation was carried out using a calibration curve prepared with pure ascorbic acid.

2.6.3. α-Tocopherol

α-Tocopherol was determined as described by Fiorentino et al. (2009). Leave flours (100 mg) were extracted with 5 mL of hexane/ethyl acetate (17:3 v/v) added with 4 μg/mL of BHT (butylated hydroxytoluene) as antioxidant. The mixture was sonicated for 1 h and filtered through cheesecloth; the residue was re-extracted one more time and the extracts mixed. The solvent was eliminated under vacuum (40 °C) with a rotary evaporator, and the residue was re-suspended in 1 mL of hexane; the sample was passed through a syringe filter (17 mm × 0.45 μm, PVDF, Titan) and analyzed with a gas chromatograph HP6890 coupled with a mass spectrometer 5973N (Agilent Technologies, USA). Separation was done in a fused silica capillary column QUADREX 007 CARBOWAX 20 M (30 m × 0.25 mm i.d., film thickness 0.25 μm) using helium as carrier gas; the flow was 0.9 mL/min for 60.5 min. Temperature conditions were: injector port at 250 °C; initial oven temperature 60 °C for 1 min, gradient at 5 °C/min to 200 °C and then at 10 °C/min to 275 °C, and held at 275 °C for the rest of analysis. A 5 μL volume sample was injected in splitless mode. Full-scan mass spectra were collected between 15 and 800 u at 2 scan/s. The MS was operated in the electron impact (EI) ionization mode with electron energy of 70 eV. The ion source and quadrupole temperatures were maintained at 245 and 150 °C, respectively. α-Tocopherol was quantitated by using a calibration curve prepared with an authentic standard.

2.6.4. Total phenolics

Total phenolics were quantitated by the Folin–Ciocalteu method as described by Singleton and Rossi (1965) with slight modifications. *Echeveria* ME solutions were prepared in methanol (*E. craigiana* and *E. kimnachii*, 1.5 mg/mL; *E. subrigida*, 1 mg/mL). *Echeveria* solution or standard (0.02 mL) was mixed with distilled water (1.58 mL) and 0.1 mL of the Folin–Ciocalteu reagent. The mixture was stirred for 2 min (40 °C/darkness) and then added with 0.3 mL of a saturated solution of Na_2CO_3; the mixture was allowed to stand at 40 °C for 30 min and then the absorbance was measured at 765 nm (Spectronic Genesis 20, Spectronic Instruments, USA). Water was used instead of sample as negative control. Quantitation was carried out by using a calibration curve of

gallic acid (0–500 μg/mL) and the results expressed as Gallic Acid Equivalents (GAE) (mg GAE/g f.w.).

2.6.5. Total flavonoids

Total flavonoids were determined for the ME of the three *Echeveria* species as reported by Quettier-Deleu et al. (2000). ME were dissolved in methanol (2 mg/mL) and an aliquot (1 mL) of each extract was mixed with 2% methanol solution of $AlCl_3 \bullet 6H_2O$ (1:1 v/v), allowed to stand for 1 h (25 °C/darkness) and the absorbance was measured at 415 nm. Quantitation was carried out by using a calibration curve of quercetin (0–50 μg/mL) and the results were expressed as Quercetin Equivalents (QE) (mg QE/g f.w.).

2.7. Determination of the antioxidant activity

2.7.1. DPPH method

The DPPH method measures the ability of substances to donate electrons or hydrogen atoms, which is associated with the bleaching of the purple colored solution of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Brand-Williams et al., 1995). For the evaluation, *Echeveria* ME were dissolved in methanol (100–400 μg/mL). Trolox solutions were used as standards. A 150 mM DPPH solution in methanol (1.8 mL) was mixed with 0.2 mL of the sample, allowed to stand for 20 min (37 °C/darkness) and the absorbance was measured at 515 nm. Before sample readings, the spectrophotometer was adjusted to zero with a mixture of 0.2 mL of the sample and 1.8 mL of methanol. A calibration curve was prepared with Trolox solutions (0–100 μg/mL) processed as described for the sample and used for quantitation as Trolox Equivalents (TE) (μmol TE/g f.w.).

2.7.2. ABTS method

In the ABTS (2,2′-azinobis-3-ethylbenzotiazoline-6-sulfonic acid) method, the reagent is oxidized to its corresponding radical $ABTS^{\bullet+}$, which shows an intense coloration in solution. The antioxidant substances transfer electrons or hydrogen atoms to the radical $ABTS^{\bullet+}$ species being inactivated and measured as the loss of color (Re et al., 1999). The $ABTS^{\bullet+}$ was prepared by mixing 5 mL of 7 mM ABTS reagent and 88 μL of potassium persulfate (140 mM) and the mixture was allowed to stand for 12–16 h at 25 °C in darkness. The $ABTS^{\bullet+}$ radical was diluted with phosphate buffer solution (PBS) (7 mM, pH 7.4) to get an absorbance of 0.700 ± 0.020 at 734 nm. In assay tubes, 0.5 mL of samples diluted in methanol (*E. craigiana* and *E. subrigida*, 100 μg/mL; *E. kimnachii*, 300 μg/mL) was mixed with 1.95 mL of the radical solution; the mixtures were allowed to stand for 10 min at 37 °C in darkness and their absorbances were measured at 734 nm. A Trolox calibration curve was prepared in the range of 0–200 μg/mL and the antioxidant activity expressed as Trolox Equivalents (μmol TE/g f.w.).

2.7.3. ORAC (oxygen radicals absorbance capacity) method

The ORAC assay measures the ability of an antioxidant, acting by hydrogen transfer mechanism, to stabilize fluorescein attacked by peroxyl radicals generated by AAPH; this activity is associated with a decreased rate in the loss of the fluorescence over time (Prior et al., 2005). Briefly, the *Echeveria* ME were dissolved in methanol (10 mg/mL) and diluted 1:500 v/v with PBS (75 mM, pH 7.4). A 96-well black microplate with flat bottom was filled with 25 μL of sample or 25 μL of PBS (blank) or 25 μL of Trolox (25–125 μM) (standard curve). The microplate was placed in the fluorescence reader (Synergy 2 SL, BioTek Instruments, USA), where 200 μL of fluorescein (0.96 μM) and 75 μL of APPH (95.8 mM) were automatically added. The fluorescence intensity (485 nm (ex)/525 nm (em)) was measured for 75 min (37 °C) with 1 min intervals (Huang et al., 2002). The antioxidant capacity was determined by contrasting the area under the curve obtained with Trolox and that obtained with our sample. Results were expressed as Trolox Equivalents (μmol TE/g f.w.).

2.7.4. β-Carotene bleaching method (β-CBM)

The β-carotene bleaching method measures the ability of an antioxidant to avoid the loss of the yellow color of a β-carotene solution due to its reaction with radicals formed by linoleic acid oxidation in an emulsion at elevated temperature (Wang et al., 2006). Briefly, the reaction mixture was prepared with 50 mg of Tween 40, 6.25 μL of linoleic acid and 500 μL of β-carotene (2 mg/mL in CH_2Cl_2); solvent was eliminated under N_2(g) and 25 mL of H_2O_2 was added and stirred vigorously to form an emulsion. A blank mixture was prepared with the same procedure but without β-carotene. Using 96 microwell plates, a blank was prepared by mixing 50 μL of DMSO and 250 μL of the blank mixture; 50 μL of the sample and 250 μL of the reaction mixture were used for sample evaluation; negative and positive controls contained 50 μL of DMSO or BHT (at the same concentrations used for the samples), respectively. Plates were incubated at 50 °C and the absorbance was measured at 492 nm immediately and at 120 min using a UV/Vis Microplate reader (Multiskan Bichromatic, Fisher Scientific, USA). Degradation rate (DR) was calculated using the equation $DR = [\ln(a/b)]/t$ (Uguzlar et al., 2012); where "a" is the absorbance at initial time, "b" is the absorbance at 120 min and "t" is the time in minutes. Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the equation $\%AA = [(DR_{control} - DR_{sample})/DR_{control}] \times 100$; where $DR_{control}$ is the degradation rate of the control, DR_{sample} is the degradation rate of the extract or of the antioxidant used as standard.

2.8. α-Glucosidase inhibition

The α-glucosidase inhibition was measured as reported by da Silva Pinto et al. (2008) with slight modifications. In a 96-well microplate, sample aliquots (50 μL) at different concentrations were mixed with 100 μL of α-glucosidase (0.5 U/mL) in phosphate buffer (0.1 M, pH 6.9); the microplate was incubated for 10 min at 37 °C (Stat Fax-2200, Awareness Technology, USA) and 50 μL of *p*-nitrophenyl-α-D-glucopyranoside in phosphate buffer (5 mM) was added to each well. Microplate was incubated (37 °C/10 min) and the absorbance was measured with a Multiskan Bichromatic Microplate reader (Fisher Scientific, USA). A solution without sample and a solution without substrate were used as blanks. Acarbose was used as positive control. The assay was carried out by triplicate and the percentage of α-glucosidase inhibition was calculated using the equation (%) = $[(A - B)/A]$ 100; where A is the control absorbance

(reaction mixture without sample) and *B* is the absorbance of the reaction mixture with the sample/standard.

2.9. Antibacterial activity

Minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) were determined by the microdilution assay in 96-well plates and plate assay, respectively (Cos et al., 2006). Strains were cultured in Petri dishes (TSA, tripticase soy agar) at 37 °C for 18–20 h; 2–5 colonies were suspended in 1 mL of 0.85% NaCl (w/v) and the density adjusted to 10^8 CFU/mL (0.5 McFarland value). Bacterial culture was diluted to 10^6 CFU/mL and 50 μL was deposited per well plus 50 μL of extract at each concentration. Gentamicin (0.125–8 μg/mL) was used as positive control, microorganisms without additives as negative control and the bacterial toxicity of solvents was also evaluated. The 96-well microplates were incubated at 37 °C for 18–20 h. After incubation, MIC corresponded to the concentration of the first well without turbidity or button formation; whereas for MBC determination, a sample of every well without growth was inoculated in a Petri dish with MacConkey or blood agar for Gram negative or Gram positive bacteria, respectively. Plates were incubated (37 °C/18–20 h) and the plate without growth obtained from the lowest extract concentration was selected as the MBC value.

2.10. Statistical analysis

One way analysis of variance and multiple contrast of means using the Fisher test (LSD, $\alpha = 0.05$) were carried out by the STATGRAPHICS Centurion XVI software (Statpoint Inc., Warrenton, VA, USA). Data were analyzed for Pearson's correlation using the PASW Statistics v. 18 software (SSPS Inc., USA). The IC_{50} values of α-glucosidase were calculated with the GraphPad Prism v. 5.03 software (GraphPad Software Inc. USA). For every experiment, evaluations were done at least by triplicate.

3. Results and discussion

3.1. Echeveria phytochemicals

The three *Echeveria* species showed six out of the eight analyzed family compounds (Table 1); alkaloids and cardiotonics were absent. The highest diversity of compounds was observed in *E. craigiana*, followed by *E. kimnachii* and *E. subrigida*. Terpenes/sterols, saponins, free anthracenics and tannins were more abundant in *E. kimnachii*, while the presence of coumarins and tannins was stronger in *E. subrigida* (Table 1). The chloroform extract of *E. leucotricha* was reported previously to contain triterpenes, flavonoids, cardiotonics, lactones, alkaloids, saponins, anthraquinones and tannins (Martínez-Ruiz et al., 2012); the composition of this extract was similar to those of our extracts and the differences found may be associated with the use of different extraction solvents. Alkaloids were absent in the three *Echeveria* species analyzed (Table 1), contrasting with its presence in *Echeveria venezuelensis* (Stevens et al., 1995).

Table 1 Phytochemical screening and yield of methanol extracts obtained from *Echeveria* plants.

Phytochemical	Methanol extract of *Echeveria*[a]		
	craigiana	*kimnachii*	*subrigida*
Yield (% d.w.)	32.3 ± 0.8	40.3 ± 1.2	20.7 ± 0.9
Terpenes/sterols	+	+ +	–
Flavonoids	+	–	+
Tannins	+	+ + +	+ + +
Saponins	+	+ +	–
Coumarins	+	+	+ +
Free anthracenics	+	+ +	–
Alkaloids	–	–	–
Cardiotonics	–	–	–

[a] (+ + +) strong, (+ +) medium, (+) poor presence, and (–) complete absence of metabolite.

3.2. Antioxidant compounds in species of Echeveria

E. subrigida showed the highest content of three out of the five analyzed components (*i.e.* ascorbic acid, total phenolics and α-tocopherol), while *E. kimnachii* and *E. craigiana* showed the highest content of β-carotene and total flavonoids, respectively (Table 2). These components have not been previously reported in *Echeveria* spp. However, *Kalanchoe diagremontiana* (another Crassulaceae) showed up to six times higher content of β-carotene (2.6 mg/100 g f.w.) (Anisimov et al., 2009) and up to 3.6 times lower values of α-tocopherol (1.7–2.5 mg/100 g f.w.) (Kruk et al., 2011) than the three studied *Echeveria* species (Table 2). *E. subrigida* showed two times more total phenolics than *Kalanchoe pinnata* (149.3 mg GAE/g ME) (Sharker et al., 2012).

Contrasting our results with those obtained for the aqueous extract of *Aloe vera*, a Crassulacean Acid Metabolism Plant (CAM) as the *Echeveria* spp., the *A. vera* extract showed higher content of ascorbic acid (42.2 mg/100 g f.w.) and β-carotene (1.5 mg/100 g f.w.) and lower content of α-tocopherol (0.147 mg/100 g f.w.), total phenolics (24.1 mg GAE/100 g f.w.) and flavonoids as Catechin Equivalents (CE) (24.5 mg CE/100 g f.w.) (Ozsoy et al., 2009).

Contrasting our results with those of other vegetables, the β-carotene contents of the three *Echeveria* species were similar to those reported for broccoli (*Brassica oleracea*) (0.414 mg/100 g f.w.), green asparagus (*Asparagus officinalis*) (0.320 mg/100 g f.w.) and artichokes (*Cynara scolymus* L.) (0.047 mg/100 g f.w.) (Beltrán et al., 2012), whereas the ascorbic acid contents were similar to those reported for some fruits such as Andean blackberry (*Rubus glaucus* Berth) (10–11 mg/100 g f.w.), grenadia (*Passiflora ligularis* L.) (16–25 mg/100 g f.w.), and naranjilla (*Solanum quitoense* Lam.) (11–13 mg/100 g f.w.) (Vasco et al., 2008). On the other hand, except for *E. craigiana*, the α-tocopherol contents of the *Echeveria* species were similar to foods rich in this nutrient: *e.g.* maize (8.1 mg/100 g f.w.) and peanuts (8.2 mg/100 g f.w.) (Wyatt et al., 1998). Total flavonoid contents of the studied *Echeveria* species were similar to those found in broccoli (*B. oleracea* L var *botrytis* L subvar *cymosa)* (31.6 mg QE/100 g f.w.) and cabbage (*B. oleracea* L var *botrytis* L)

Table 2 Component quantitation and antioxidant activities of *Echeveria* plants.[a]

Echeveria species	β-Carotene	Ascorbic acid	α-Tocopherol	Total phenolics[b]	Total flavonoids[c]
		mg/100 g f.w.		mg GAE/100 g f.w. (mg GAE/g ME)	mg QE/100 g f.w. (mg QE/g ME)
E. craigiana	0.31 ± 0.02^{x}	10.4 ± 1.4^{x}	2.9 ± 0.5^{x}	152.2 ± 7.0^{x} (102.1 ± 4.7)	**36.4 ± 1.3^{x} (24.4 ± 0.9)**
E. kimnachii	**0.43 ± 0.02^{y}**	10.3 ± 1.2^{x}	7.6 ± 0.8^{y}	153.7 ± 12.3^{x} (108.8 ± 8.7)	17.9 ± 1.8^{y} (12.7 ± 1.3)
E. subrigida	0.043 ± 0.002^{z}	**16.7 ± 1.6^{y}**	**9.0 ± 1.3^{y}**	**400.5 ± 9.6^{y} (302.8** ± 7.3)	31.6 ± 3.4^{z} (23.9 ± 2.6)

[a] Values are the mean ± standard deviation of three replicates and those in bold type shows the species with the highest content; f.w. stands for fresh weight basis and ME for methanol extract. Different letters in the same column (x, y, z) showed significant differences ($P \leqslant 0.05$).
[b] Phenolics content was expressed as Gallic Acid Equivalents (GAE).
[c] Flavonoid content was measured as Quercetin Equivalents (QE).

(17.2 mg QE/100 g f.w.) (Bahorun et al., 2004). The total phenolics of the three *Echeveria* species were high and similar to those reported for bitter melon (*Momordica charantia* L.) (143.6 mg GAE/100 g f.w.), and highbush blueberry (*Vaccinium corymbosum L.* Hybrids) (399.3 mg GAE/100 g f.w.) (Lin and Tang, 2007; Sellappan et al., 2002).

3.3. Antioxidant activity

The highest antioxidant activities of the ME were for *E. craigiana* (DPPH and ABTS) and *E. subrigida* (ORAC and β-CBM). The activity of *E. kimnachii* by the β-CBM was similar to that of *E. subrigida* (Table 3). ABTS values were about two times higher than DPPH values, perhaps by the additive effect of hydrophilic and lipophilic components detected by the ABTS method (Floegel et al., 2011). The pertinence of using several antioxidant methods is supported by the differences found in the activities evaluated by ABTS, DPPH, ORAC and β-CBM, which differ in some chemical principles and capability to detect antioxidants of different polarity.

The antioxidant activities of the *Echeveria* ME were similar to those of fruits recognized by their antioxidant capacity such as berries (Paredes-López et al., 2010). The ABTS values for the three *Echeveria* species were higher than those of highbush blueberry (*V. corymbosum*) (14.0 μmol TE/g f.w.) and strawberry (*Fragaria x. ananassa*) (11.9 μmol TE/g f.w.); whereas DPPH and ORAC results were similar to those of raspberry (*Rubus idaeus*) (25.3 μmol TE/g f.w.) and lingonberry (*Vitis vitis-idea*) (31.8 μmol TE/g f.w.), respectively (Paredes-López et al., 2010).

The ME of the three *Echeveria* species showed high activity by the β-CBM, inactivating the free radicals derived from linoleic acid oxidation. The antioxidant values were slightly lower than those obtained for the commercial antioxidant BHT (86.6%) and raspberry (*R. idaeus*) (86.0%, BHA 88.1%), and higher than that of highbush blueberry (*V. corymbosum*) (19.5%, BHT 83%) (Rodrigues et al., 2011; Tosun et al., 2009). Thus, *E. subrigida* and *E. kimnachii* are species with high antioxidant capacities (> 70%) (Hassimotto et al., 2005).

Total antioxidant activities of the analyzed samples are associated with the combined effect of phytochemicals acting by additive or synergic effects. Some phytochemicals, such as phenolics, show a higher contribution and their content correlate positively with the antioxidant activity (Teow et al., 2007). For the studied *Echeveria* species, antioxidant activities were correlated with hydrophilic components for the DPPH and ABTS (flavonoids) and ORAC (phenolics and ascorbic acid), as well as with lipophilics for the β-CBM (α-tocopherol) (Table 4). However, the antioxidant activities of the different methods did not correlate ($P \geqslant 0.16$); the highest correlation value was between DPPH and ABTS methods ($r = 0.97$, $P = 0.16$).

The correlation between antioxidant activity and the content of total phenolics and ascorbic acid is commonly reported (Du et al., 2009; Velioglu et al., 1998). Moreover, the lack of correlation between these components and the β-CBM was also reported by Hassimotto et al. (2005). Interestingly, the correlation between the content of α-tocopherol and antioxidant activity by the β-CBM has not been previously reported; our results supported that α-tocopherol (lipophilic) must be better antioxidant in lipophilic media and consequently better

Table 3 Antioxidant activity of methanol extracts of *Echeveria* species.[a]

Echeveria species	Antioxidant activity method			
	DPPH	ABTS	ORAC	β-Carotene bleaching
	μmol TE/g f.w. [b]			% *AA*[c]
E. craigiana	26.19 ± 0.24^{x}	65.91 ± 2.58^{x}	32.52 ± 1.48^{x}	68.5 ± 1.2^{x}
E. kimnachii	12.01 ± 0.63^{y}	23.36 ± 0.14^{y}	32.25 ± 1.91^{x}	76.7 ± 1.5^{y}
E. subrigida	24.83 ± 0.88^{z}	51.58 ± 1.16^{z}	37.58 ± 2.63^{y}	79.3 ± 2.3^{y}

[a] Values are the mean ± standard deviation of at least three measurements. Different superscript letters (x, y, z) in the same column show statistical differences ($P < 0.05$).
[b] Antioxidant activities were reported as micromoles of Trolox Equivalents in fresh weight basis (μmol TE/g f.w.).
[c] Extract concentration was 500 μg/mL and results were expressed as percentage of antioxidant activity (% *AA*), BHT was used as standard (% *AA* = 86.6 ± 1.9).

Table 4 Pearson's correlation coefficient between *Echeveria* components and antioxidant activity of methanol extracts of *Echeveria* species.[a]

Echeveria component	Evaluation method of the antioxidant activity			
	DPPH	ABTS	ORAC	β-CBM
β-Carotene	−0.66 (0.53)	−0.47 (-0.69)	−0.97 (0.16)	−0.44 (0.71)
Ascorbic acid	0.42 (0.72)	0.20 (0.88)	**1.00** (0.02)	0.68 (0.52)
α-Tocopherol	−0.38 (0.75)	−0.60 (0.59)	0.64 (0.56)	**1.00** (0.01)
Total phenolics	0.42 (0.73)	0.18 (0.89)	**1.00** (0.03)	0.69 (0.51)
Total flavonoids	0.99 (0.11)	**1.00** (0.05)	0.31 (0.8)	-0.52 (0.65)

[a] In parentheses are presented the *P* values. Bold type numbers showed significant correlation ($P \leqslant 0.05$).

detected by the β-CBM; tocopherols, BHT and BHA are better antioxidants than phenolic acids and flavonoids by this method (Ouchikh et al., 2011; Von Gadow et al., 1997).

The results indicate that the evaluated *Echeveria* species are excellent sources of natural antioxidants, mainly phenolics and α-tocopherol, and they could be exploited for food and pharmaceutical uses.

3.4. α-Glucosidase inhibition

The inhibition of digestive enzymes (*e.g.* α-amylase and α-glucosidase) is suggested as an efficient strategy to control the blood glucose levels; consequently, these inhibitors are potential antidiabetic agents (De Luca et al., 2012; Gulati et al., 2012). Remarkably, the ME of the studied *Echeveria* species showed 63–143 times better α-glucosidase inhibition values than the commercial drug acarbose (IC_{50} 3.59 ± 1.03 mg/mL) with the following values and order *E. subrigida* (IC_{50} 25.21 ± 3.66 μg/mL) > *E. craigiana* (IC_{50} 56.67 ± 4.2 μg/mL) > *E. kimnachii* (IC_{50} 50.57 ± 1.48 μg/mL). This is the first report of β-glucosidase activity of any *Echeveria* spp.

The Crassulaceae genera *Sedum* and *Kalanchoe* are traditionally used against diabetes. The antidiabetic activity of *Sedum dendroideum* (Spanish common name, *e.g.*, siempreviva) and *K. pinnata* (Lam.) Pers (Spanish common name, *e.g.*, tronador) has been demonstrated in a mouse model and associated with sedoheptulose and flavonoids, respectively (Andrade-Cetto and Heinrich, 2005). Moreover, the number of papers registering α-glucosidase inhibitory activity of plant extracts has recently increased. In general, the best extracts show IC_{50} values two times higher than acarbose and lower values were only registered for pure compounds derived from such extracts (Ali et al., 2013; Kang et al., 2012; Moradi-Afrapoli et al., 2012); however, hydroalcoholic extracts of two plants from Benin used to treat diabetes show better activity than acarbose (IC_{50} 726 μg/mL) (*Polygonum senegalensis*, 1.5 μg/mL; *Pseudocedrela kotschyi*, 5 μg/mL) (Moradi-Afrapoli et al., 2012), as well as the aqueous extract of *Brickellia cavanillesii* (IC_{50}, extract 0.169 mg/mL and acarbose 1.12 mg/mL) (Escandón-Rivera et al., 2012). Remarkably, the ME of the three *Echeveria* species used in the present study were more active than acarbose and these plants could be promising sources of hypoglycemic compounds. *E. subrigida* showed the highest content of total phenolics (Table 2) which could be associated with its highest inhibition of α-glucosidase, but further studies are required to elucidate whether or not phenolics are responsible for this activity.

3.5. Antibacterial assay

The order for the antibacterial activity of the studied *Echeveria* ME were *E. subrigida* > *E. kimnachii* > *E. craigiana*; all Gram positive strains were inhibited being *S. aureus*

Table 5 Minimum Inhibitory Concentration (MIC) of the bacterial growth by *Echeveria* methanol extracts.

Microorganisms	MIC (μg/mL)[a]			
	E. craigiana	*E. kimnachii*	*E. subrigida*	Gentamicin
Gram-positive				
Staphylococcus aureus ATCC 29213	500	125	62.5	1
Streptococcus group A-4	1000	1000	125	0.5
Staphylococcus aureus 3	125	31.25	15.63	0.5
Gram-negative				
Escherichia coli ATCC 25922	–	–	–	1
Escherichia coli AO11	250	125	62.5	0.5
Salmonella Typhi	–	–	–	1
Shigella dysenteriae	1000	1000	125	0.5

"–" Stands for no activity up to such concentration.
[a] Antibacterial activity assayed up to 1000 μg/mL.

the most sensitive. The antibacterial activity of the ME of the three *Echeveria* species was only observed for two out of the four evaluated Gram negative strains (Table 5). The gentamicin MIC values for the ATCC strains corresponded with those reported previously (Lennette et al., 1987). The three *Echeveria* species ME were not bactericide at the evaluated concentrations (⩽1000 μg/mL).

Gram positive bacteria are usually more sensitive to plant extracts than Gram negative strains as observed in this report; this phenomenon has been associated with the cell wall structural differences between these two groups of bacteria (Sharma et al., 2012).

S. aureus is a common nosocomial infectious agent and a continuous public health concern because of its ability to acquire resistance; *e.g.* methicillin-resistant *S. aureus* (MRSA) shows resistance against most of the commercial antibiotics including some developed more recently such as vancomycin, linezolid (oxazolidinone derivative) and streptogramin quinupristin/dalfopristin mixture (Gibbons, 2004), emphasizing the importance of discovering new antibacterial agents. The studied *Echeveria* ME were active against *S. aureus* as it was previously registered for the ME obtained from the aerial parts of *E. leucotricha* (MIC = 20 μg/mL *vs.* methicillin resistant strain); this MIC value was similar to those obtained in the present study for the ME of *E. kimnachii* and *E. subrigida* against *S. aureus* 3 (Table 5). The ME of *Bryophyllum pinnatum* (Crassulaceae) leaves were active against Gram positive bacteria, particularly for *S. aureus* (ATCC 25213 and an isolated strain) (MIC = 32 mg/mL) (Akinsulire et al., 2007), but our extracts were much better. In another study, the ethanol extract of *B. pinnatum* showed activity against *S. aureus* ATCC 25922 (MIC 256 μg/mL) and *S. enterica* serovar Typhi ATCC 6539 (64 μg/mL) (Tatsimo et al., 2012); the MIC values of the *Echeveria* ME against the *S. aureus* control strain (62.5–500 μg/mL) corresponded with the value reported for the *B. pinnatum* ME.

The antibacterial activity of the *B. pinnatum* was associated with kaempferol type compounds (MIC = 1–256 μg/mL) (Tatsimo et al., 2012). Our phytochemical results suggested that phenolics, tannins and coumarins were associated with the antibacterial activity, as these compounds have showed this activity (Martínez-Ruiz et al., 2012; Sharma et al., 2012).

Considering the low MIC values obtained for the three *Echeveria* species ME against *S. aureus* (15.63–500 μg/mL) and in particular of *E. subrigida* against every sensitive strain (15.63–125 μg/mL), values which are much lower than the reference one of 1000 μg/mL, these plants could be a source of clinical relevant antibacterial compounds (Gibbons, 2004).

4. Conclusions

We are reporting for the first time that three *Echeveria* species (*E. kimnachii, E. craigiana* and *E. subrigida*) are good sources of antioxidants (*e.g.* α-tocopherol and phenolics) and that their methanol extracts show several biological activities (*i.e.* antioxidant, α-glucosidase inhibition and antibacterial). Thus, preparations or compounds of these plants could be useful for the prevention/treatment of some of the most important causes of morbidity and mortality (*i.e.* infectious and chronic degenerative diseases, and particularly diabetes), and future studies are warranted to support such uses.

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