academicJournals

Vol. 10(11), pp. 1203-1210, 12 March, 2015
DOI: 10.5897/AJAR2014.9253
Article Number: 4374C3451399
ISSN 1991-637X
Copyright ©2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Free radical scavenging properties and their relationship with bioactive compounds content of dehydrated calyces of roselle (*Hibiscus sabdariffa* L.)

Medina-Carrillo, Raquel Enedina[1], Madrigal-Santillán, Eduardo[2], Machuca-Sánchez, María Luisa[3], Balois-Morales, Rosendo[3], Jiménez-Ruiz, Edgar Iván[3], Valadez-Vega, Carmen[4], Morales-Gonzalez, Jose Antonio[2] and Sumaya-Martínez, María Teresa[3]*

[1]Posgrado en Ciencias Biológico Agropecuarias, Universidad Autónoma de Nayarit. Carretera Tepic-Compostela km. 9. Xalisco, Nayarit, 63780, México.
[2]Escuela Superior de Medicina, Instituto Politécnico Nacional. Plan de San Luis y Díaz Mirón s/n, Unidad Casco de Santo Tomas. México, D.F. 11340, México.
[3]Secretaría de Investigación y Posgrado, Universidad Autónoma de Nayarit, Ciudad de la Cultura "Amado Nervo", Boulevard Tepic-Xalisco S/N. Tepic, Nayarit, Mexico.
[4]Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo. Ex-Hacienda de la Concepción. Tilcuautla, Pachuca de Soto, Hidalgo, 42080, México.

Received 18 October, 2014; Accepted 11 March, 2015

Roselle (*Hibiscus sabdariffa* L.) also called roselle fruit or flower of jamaica is a plant used in the traditional medicine due to its wealth of bioactive compounds. These compounds confer beneficial health benefits on it in aqueous infusions prepared with the blossoms of the jamaica flower. In the present study, we determined the antioxidant activity of 64 roselle varieties and quantified the following bioactive compound contents: phenolic; monomeric anthocyanins, and ascorbic acid. The results show that highest antiradical scavenging activity and reductor capacity belonged to varieties with dark-red calyces. Similarly, we found that the bioactive compound concentration increased as the pigmentation of the fresh calyces intensified. Finally, our results demonstrated that the aqueous extracts' antioxidant activity is correlated with the bioactive compound concentration, this correlation greater with the content of ascorbic acid.

Key words: Roselle, antioxidant activity, phenolic compounds, monomeric anthocyanins.

INTRODUCTION

Bioactive compounds promote beneficial effects on health, and among these we find their action as antioxidants. Free radicals (FR) are substances generated by the aerobic metabolism, reactions with

*Corresponding author. E-mail: teresumaya@hotmail.com. Tel: +52 (311) 2 11 88 51.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

drugs or with environmental agents. When the cellular levels of these species overcome an organism's antioxidant defense system, a condition appears that is denominated oxidative stress (OS), which can cause cell damage, trigger physiological disorders, and favor the appearance of health problems such as cancer, cardiovascular diseases, and degenerative and inflammatory diseases. Bioactive compounds are the product of plant-related metabolism and are characterized by promoting beneficial effects in an organism; among these effects, we find inhibiting or delaying the reaction of FR on biological structures, inhibiting lipid peroxidation, and chelating heavy metal ions, and examples of bioactive compounds comprise phenolic compounds, pigments, and vitamins. Antioxidant substances represent one of the important mechanisms of defense against Free radicals (FR), but Endogenous Antioxidant Molecules (EAM) by themselves are not sufficiently effective for counteracting the damage caused by reactive oxygen species, particularly if the present lifestyle is taken into account: Smoking, drugs, alcohol, unbalanced diet, contamination and inadequate solar exposure, all of which facilitate the formation of Free radicals (FR). Thus, increasing the dietary intake of antioxidants is of great importance for good health, as evidenced by studies that characterize the antioxidant activity of foods (Gregoris et al., 2013). At present, there is great interest in consuming antioxidants derived from natural sources, such as plants that have been utilized in traditional medicine due to their being rich in bioactive compounds (Mungole and Chaturvedi, 2011), and roselle (*Hibiscus sabdariffa* L.*)* is one of those cases. The calyces and flowers of roselle, or *jamaica*, as it is known in Spanish, contain alkaloids, ascorbic acid, β-carotene, citric acid, malic acid, protocatechuic acid, anthocyanins, quercetin, pectin, and polysaccharides. In addition, the extracts obtained from the calyces contain phytochemical compounds such as polyphenic acids, flavonoids, and anthocyanins (Maganha et al., 2010), which confer upon them properties that are beneficial for health, including antihypertensive and hypocholesterolemic characteristics (Hopkins et al., 2013), antibacterial properties (Yin and Chao, 2008), selective cytotoxic and apoptogenic activity (Khaghani, 2011), and even anticancerigenous activity (Lin et al., 2012). Anthocyanins present in the extracts confer on the latter 51% of their antioxidant activity (Tsai et al., 2002). The objective of this work was to characterize the antioxidant activity and concentration of the bioactive compounds of 64 varieties of roselle (*jamaica*).

MATERIALS AND METHODS

The following reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): 1,1-Diphenyl-2-picrylhydrazyl (DPPH•); 2,6-Dichloro-indophenol (DCPI); sodium acetate; Ascorbic acid (AA), gallic acid; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); ferric chloride(III); potassium chloride; potassium hexacyanoferrate(III); potassium persulfate; Folin-Ciocalteu reagent; glacial acetic acid; oxalic acid; trichloroacetic acid; 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•$^+$); sodium carbonate, and anhydrous ethanol. A spectrophotometer UV-Vis BioTek PowerWave XS with microplates reader was used to measure absorbances in all techniques.

Experimental determinations

Reception and sample preparation

We received 64 dehydrated calyces of varieties of roselle (*Hibiscus sabdariffa* L.) that had been cultivated in the experimental fields of the Academic Unit of Agriculture belonging to the Autonomous University of Nayarit (UAN), Mexico, during the period of July through December 2012. The dehydrated calyces were frozen at -18°C for their later lyophilization to constant weight. Once they were dried, they were ground until a fine powder was obtained. We performed aqueous extractions for each of the varieties, in triplicate, following the method proposed by Prenesti et al. (2007). The aqueous extract was stored at -18°C until time for its analysis. For determination of total monomeric anthocyanins, we followed the protocol described by Giusti and Wrolstad (2005).

DPPH radical scavenging activity

DPPH●-based antiradical activity was evaluated according to the procedure reported by Morales and Jiménez-Pérez (2001). The technique consisted of preparing a solution of DPPH● at a concentration of 74 mg·L^{-1} in ethanol and shaking it for 10 min. Later, we placed 100 µl of the samples in vials, added 500 µl of DPPH● solution, and shook vigorously; these vials were allowed to stand at room temperature during 1 h. After this time, the vials were centrifuged at 13,000 *g* for 5 min at room temperature and later, supernatant absorbance was measured at a wavelength of 520 nm. The scavenging capacity of the free radical DPPH• of the aqueous extracts was obtained from a standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and was expressed as Trolox-equivalent (TE) micromoles per gram of calyces dry base (d.b.) (µmol TE·g^{-1} calyces d.b.).

ABTS•$^+$ radical scavenging activity

ABTS•$^+$ stable cation radical scavenging capacity was determined according to the method reported by Re et al. (1999) and by Kuskoski et al. (2005). The ABTS•$^+$ radical is obtained from the reaction of ABTS•$^+$ (7 mmol·l^{-1}) with potassium persulfate (2.45 mmol·l^{-1}) at a 1:0.5 ratio (v/v) and incubated at 4°C under conditions of darkness during 16 h. Once formed, the ABTS•$^+$ radical is diluted with ethanol until obtaining an absorbance value of between 0.70 (±0.1) and 754 nm. The samples (including the standardized curve) are adequately diluted with water until 20 to 80% inhibition of the initial ABTS•$^+$ color is produced. Twenty microliter of the sample is added to 980 µl of the ABTS•$^+$ dilution. Absorbance is measured at 754 nm at 7 min. The radical scavenging capacity of the free radical ABTS•$^+$ of the extracts was determined by means of a standardized curve of AA and was expressed as TE of Ascorbic acid (AA) per g dry base (d.b.) calyces (mg EAA·g^{-1} calyces d.b.).

Determination of the reduction capacity of the Fe(III) ion into Fe(II)

We determined this by the method of Hinneburg et al. (2006). The

method consisted of taking a 25 µl aliquot of each extract; mixed this with 63 µl of phosphate buffer (0.2 $mol \cdot l^{-1}$, pH 6.6) and 63 µl of potassium hexacyanoferrate (III) [$K_3Fe(CN)_6$] at 1%. After to 30 min incubation at 50°C, we added 63 µl of chloroacetic acid at 10% and centrifuged this during 10 min at 13,000 *g*. Next, we added 63 µl of supernatant to 63 µl of water and 12.5 µl of ferric chloride. Absorbance was registered at 700 nm. The capacity to reduce the iron (III) was determined as mg acetic acid equivalents (AAE) per gram of dry base (d.b.) calyces (mg $AAE \cdot g^{-1}$ of calyces d.b.) from a standard curve of AA.

Determination of the concentration of total phenolic compounds

This was determined according to the method of Stintzing et al. (2005), which utilizes the Folin-Ciocalteu reagent. The blue coloration is read at a wavelength of 765 nm and reflects the total amount of polyphenols, expressed as gallic acid equivalents (GAE). One hundred microliter of the sample was measured and we added 500 µl of Folin-Ciocalteu solution (1:10 in deionized water) and 400 µl of sodium carbonate solution (at 7.5%); then, the samples were vortex-shaken and incubated at room temperature during 30 min. Afterward, we measured absorbance at a wavelength of 765 nm. Total phenolic compound concentration was obtained from a standardized curve of gallic acid and was expressed as gallic acid equivalents (GAE) per gram of dry base (d.b.) calyces (mg $GAE \cdot g^{-1}$ of calyces d.b.).

Determination of the concentration of total monomeric anthocyanins

Total monomeric anthocyanin content was determined by spectrophotometry according to the protocol described by Giusti and Wrolstad (2005), which utilized the differential pH. For analysis of the total monomeric anthocyanin content, we placed 50 µl aliquots of the filtered solution into two assay tubes and measured this with 450 µl of pH 1.0 and pH 4.5 buffer solutions, respectively (Dilution factor [DF] = 10). In both solutions, we measured absorbance at 520 and 700 nm wavelengths by Ultraviolet (UV)-Vis spectrophotometry. The content of the pigment was calculated as delphinidin-3-glucoside with the following formula:

$$CA = \frac{A * MW * DF * 1000}{£ * 1}$$

in which CA = Concentration of total monomeric anthocynanins, mg delphinidin-3-glucoside (D3G) per liter, DF = Dilution factor, A = Absorbance, (A_{520} pH 1 – A_{700} pH 1) – (A_{520} pH 4.5 – A_{700} pH 4.5), l = length of the passage of light in a cell in cm (0.64 cm), £ = coefficient of molar absorbance 27,481 $l \cdot (mol \cdot cm)^{-1}$ for delphinidin-3-glucoside, and MW = Molecular weight of delphinidin-glucoside (465.2 $g \cdot mol^{-1}$). Total monomeric anthocyanins concentration was expressed as mg delphinidin-3-glucoside (D3G) per gram of dry base (d.b.) calyces (mg $D3G \cdot g^{-1}$ of calyces d.b.).

Determination of ascorbic acid concentration

We employed the colorimetric method described by Dürüst et al. (1997)and prepared the following solutions: 2,6 Dichlorophenol-indophenol (DCPI) disodic salt at 24 mg·L of distilled water, oxalic acid at 0.4% in distilled water, acetate buffer (composed of 3 g of sodium acetate, 7 ml of distilled water, and 10 ml of glacial acetic acid), and a base solution of 100 mg·L of ascorbic acid diluted in oxalic acid at 0.4%, to 100 µl of each of the samples plus 100 µl of acetate buffer and 800 µl of DCPI. After vortex-shaking of the samples, absorbance was read at 520 nm, utilizing as target oxalic acid at 0.4%. The ascorbic acid (AA) concentration in the extracts was determined by means of a standard curve and was reported as mg of AA per g calyces of dry base (mg $AA \cdot g^{-1}$ calyces d.b.).

Statistical analysis

The results obtained of the different determinations were analyzed by determining their normality and homoscedasticity. After this, we carried out Analysis of variance (ANOVA) test by means of Tukey test ($p < 0.05$) for each of the determinations. We established correlations between the concentrations of bioactive compounds (total phenols, total monomeric anthocyanins, and ascorbic acid) and determinations of antioxidant activity. The data were compiled and organized employing Microsoft Excel 2010 and for statistical analysis, we utilized the Minitab ver. 16 program.

RESULTS AND DISCUSSION

Concentrations of bioactive compounds and determinations of the antioxidant activity of the aqueous extracts of the varieties analyzed, grouped subjectively by the color of the fresh calyces, are depicted in Table 1. We observed that, even with variations in the color groups, varieties in groups with greater pigmentation presented a greater concentration of anthocyanins than those that were less pigmented. Antioxidant activity, determined as free radical scavenging capacity and reductor activity, is directly proportional to the concentration of the bioactive compounds quantified: total monomeric anthocyanin phenols and AA.

The 64 varieties of roselle analyzed were classified into groups according to concentration of bioactive compounds. The results of this classification show that there is a positive correlation between the concentration of bioactive compounds quantified and the antioxidant activity of the aqueous extracts of roselle varieties analyzed.

Determination of antioxidant activity (by the three techniques employed), phenolic content, and that of the monomeric anthocyanins of the aqueous extracts of the roselle varieties analyzed show that the red varieties present greater activity and concentration of bioactives, while green varieties present less activity. The latter coincides with that reported by Juliani et al. (2009), who analyzed phenolic compounds, $ABTS^{\bullet +}$ cation scavenging capacity, and anthocyanin content in roselle varieties of different colors in Senegal, and the results of Christian and Jackson (2009), who determined the content of anthocyanins, phenolics, and the activity on DPPH• of three roselle varieties (dark red, red, and green). With regard to the aqueous extracts' AA content, the higher concentration is found in varieties with dark-toned calyces (red and purple) and the lower one, in the green-colored varieties; this differs significantly with that previously reported by Salinas-Moreno et al. (2012) and

Babalola et al. (2001); both investigations concluded that light-toned calyces contained more AA than dark-toned

Table 1. Antioxidant activity and concentration of the bioactive compounds of 64 varieties of roselle (*jamaica*)

Variety	DPPH• [μmol TE·g^{-1} calyces d.b.]	ABTS•$^{+}$ [mg AAE·g^{-1} calyces d.b.]	FRAP [mg AAE·g^{-1} calyces d.b.]	TPC [mg GAE·g^{-1} calyces d.b.]	TMA [mg D3G·g^{-1} calyces d.b.]	AA [mg AA·g^{-1} calyces d.b.]
Green varieties						
UAN 4	68.1 ± 1.6	10.1 ± 0.1	10.4 ± 0.0	17.3 ± 0.5	0.0 ± 0.0	1.5 ± 0.1
UAN 16_1	52.2 ± 1.4	7.4 ± 0.2	9.2 ± 0.2	11.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.1
Pink variety						
$8Q_8$	37.4 ± 0.3	5.9 ± 0.0	6.0 ± 0.1	8.9 ± 0.0	0.3 ± 0.0	1.0 ± 0.0
Red varieties						
Tempranilla flor	65.3 ± 2.1	8.8 ± 0.1	7.8 ± 0.2	8.5 ± 0.0	2.6 ± 0.1	2.1 ± 0.1
Colima	76.3 ± 0.8	12.4 ± 0.4	11.8 ± 0.4	13.9 ± 0.3	4.4 ± 0.0	3.6 ± 0.1
Criolla roja	100.0 ± 1.1	13.0 ± 0.6	12.4 ± 0.2	16.4 ± 0.3	4.6 ± 0.0	3.6 ± 0.0
Criolla rojo violeta	75.1 ± 0.6	12.5 ± 3.0	9.0 ± 0.1	16.7 ± 0.0	4.0 ± 0.0	3.0 ± 0.0
Criolla super precoz	57.2 ± 1.2	6.9 ± 0.1	6.8 ± 0.0	7.7 ± 0.8	1.9 ± 0.0	1.9 ± 0.0
Criolla Puebla precoz	76.5 ± 0.0	11.8 ± 0.2	9.2 ± 0.1	10.1 ± 0.1	4.4 ± 0.2	3.1 ± 0.0
Criolla precoz	72.2 ± 3.1	12.8 ± 0.6	11.0 ± 0.1	12.3 ± 0.4	3.8 ± 0.1	3.0 ± 0.1
UAN 6 Puga	84.0 ± 0.8	13.4 ± 0.0	14.8 ± 0.0	11.4 ± 0.4	5.5 ± 0.1	3.5 ± 0.2
UAN 6 Novillero	68.3 ± 0.8	10.9 ± 0.1	13.8 ± 0.0	13.4 ± 0.1	3.6 ± 0.0	3.0 ± 0.0
UAN 25_1	66.7 ± 0.5	12.3 ± 0.2	13.4 ± 0.3	14.0 ± 0.0	3.8 ± 0.0	3.5 ± 0.1
UAN 7	145.4 ± 6.1	11.8 ± 0.3	15.8 ± 0.0	10.7 ± 0.3	5.9 ± 0.1	4.8 ± 0.1
Tempranilla roja	68.7 ± 3.2	6.9 ± 0.8	10.6 ± 0.3	10.9 ± 0.0	3.0 ± 0.0	1.9 ± 0.2
UAN 11	85.5 ± 0.3	14.1 ± 0.1	13.0 ± 0.0	15.6 ± 0.3	4.2 ± 0.0	4.1 ± 0.0
UAN 8	76.1 ± 0.4	14.5 ± 0.3	15.9 ± 0.3	14.4 ± 0.4	4.0 ± 0.0	4.1 ± 0.0
UAN 27	125.0 ± 1.2	19.7 ± 0.3	15.3 ± 0.1	13.1 ± 0.4	12.5 ± 0.1	5.7 ± 0.1
UAN 15	69.5 ± 0.6	10.5 ± 0.1	9.3 ± 0.2	10.7 ± 0.1	3.9 ± 0.0	3.1 ± 0.0
UAN 12	79.0 ± 2.4	11.4 ± 0.2	10.7 ± 0.2	14.5 ± 0.1	4.2 ± 0.0	3.4 ± 0.0
UAN 22	77.0 ± 1.3	11.2 ± 0.0	10.0 ± 0.1	13.2 ± 0.1	3.4 ± 0.1	3.1 ± 0.1
UAN 10_1	99.1 ± 1.0	13.3 ± 0.4	10.2 ± 0.5	19.0 ± 0.3	3.7 ± 0.1	3.6 ± 0.1
UAN 25	55.7 ± 1.1	10.1 ± 0.4	8.0 ± 0.4	8.3 ± 0.3	3.7 ± 0.0	2.6 ± 0.1
UAN 30	82.8 ± 0.6	11.5 ± 0.2	10.2 ± 0.2	14.0 ± 0.2	3.6 ± 0.0	3.2 ± 0.0
UAN 9	70.0 ± 1.4	9.3 ± 0.3	7.7 ± 0.3	10.4 ± 0.3	3.7 ± 0.0	3.3 ± 0.1
UAN 16	81.3 ± 2.0	12.6 ± 0.4	10.4 ± 0.1	15.6 ± 0.5	4.3 ± 0.0	3.1 ± 0.0
UAN 10_2	86.5 ± 3.4	13.8 ± 0.3	12.0 ± 0.4	11.5 ± 0.6	3.6 ± 0.0	3.0 ± 0.0
$2Q_2$	79.6 ± 0.4	13.4 ± 0.4	10.4 ± 0.2	10.1 ± 0.1	3.9 ± 0.0	3.7 ± 0.1
11 Coneja	78.1 ± 0.5	12.1 ± 0.1	9.2 ± 0.0	15.0 ± 0.2	3.7 ± 0.1	2.9 ± 0.0
$Q_{12}CR$	74.3 ± 0.1	8.3 ± 0.3	8.8 ± 0.0	12.4 ± 0.3	4.1 ± 0.0	3.2 ± 0.1
Red purple varieties						
Criolla Huajicori	74.3 ± 3.9	11.0 ± 0.2	9.5 ± 0.0	11.3 ± 0.2	3.9 ± 0.0	3.1 ± 0.1
Negra UAN	92.7 ± 3.1	12.7 ± 1.4	11.8 ± 0.2	14.8 ± 0.1	11.0 ± 0.1	5.1 ± 0.0
UAN 5	127.9 ± 0.0	11.8 ± 0.2	15.8 ± 0.7	18.1 ± 0.2	12.0 ± 0.1	6.3 ± 0.0
UAN 6_1	92.0 ± 3.6	12.2 ± 0.2	15.0 ± 0.1	14.8 ± 0.2	4.5 ± 0.2	3.3 ± 0.1
UAN 16_2	130.9 ± 0.5	19.8 ± 0.3	19.8 ± 0.2	18.8 ± 0.2	9.2 ± 0.1	5.9 ± 0.0
Bellotuda	78.9 ± 1.9	15.0 ± 0.1	13.5 ± 0.1	12.5 ± 0.2	5.1 ± 0.0	3.6 ± 0.1
UAN 23	83.7 ± 0.1	14.0 ± 0.0	14.6 ± 0.0	13.7 ± 0.5	5.1 ± 0.1	3.7 ± 0.1
UAN 24	96.2 ± 2.4	14.7 ± 0.9	14.3 ± 0.0	15.3 ± 0.8	6.7 ± 0.1	4.7 ± 0.1
UAN 13	79.2 ± 1.7	16.8 ± 0.8	13.2 ± 0.1	12.4 ± 0.0	6.1 ± 0.1	2.0 ± 0.0
UAN 26	93.3 ± 2.1	14.1 ± 0.3	10.4 ± 0.1	13.6 ± 0.2	6.9 ± 0.0	4.2 ± 0.0
UAN 23_1	94.3 ± 0.4	14.2 ± 0.2	10.0 ± 0.4	13.8 ± 0.1	5.3 ± 0.0	4.7 ± 0.0

Table 1. Contd.

UAN 13_1	67.4 ± 1.3	11.6 ± 0.4	9.4 ± 0.1	11.6 ± 0.6	3.4 ± 0.0	2.8 ± 0.0
UAN 24_1	95.5 ± 0.2	11.0 ± 0.2	9.6 ± 0.5	15.5 ± 0.2	6.6 ± 0.0	4.1 ± 0.1
UAN 20	92.3 ± 0.5	13.7 ± 0.1	12.2 ± 0.0	15.7 ± 0.1	6.1 ± 0.0	4.4 ± 0.1
UAN 29	142.9 ± 2.8	23.8 ± 0.3	17.0 ± 0.5	15.6 ± 0.5	11.2 ± 0.1	5.9 ± 0.1
UAN 19	86.9 ± 1.1	12.3 ± 0.1	10.9 ± 0.0	15.0 ± 0.2	3.8 ± 0.0	3.0 ± 0.1
$2MQ_2$	103.3 ± 0.8	14.2 ± 0.5	12.8 ± 0.2	10.2 ± 0.1	8.8 ± 0.0	5.0 ± 0.0
$4Q_4$	135.4 ± 1.8	21.8 ± 0.3	15.0 ± 0.0	18.8 ± 0.0	10.2 ± 0.1	4.7 ± 0.1
$7Q_7$	134.1 ± 3.1	22.9 ± 0.1	14.4 ± 0.8	19.3 ± 0.1	10.6 ± 0.2	6.5 ± 0.0
Variedad 10	132.9 ± 2.4	20.4 ± 0.1	15.1 ± 0.1	16.3 ± 0.0	9.8 ± 0.0	5.6 ± 0.1
Purple varieties						
Criolla morada	95.1 ± 0.9	12.2 ± 0.6	10.3 ± 0.1	15.9 ± 0.1	6.5 ± 0.0	4.8 ± 0.1
UAN 31	92.3 ± 2.3	14.1 ± 0.1	13.8 ± 0.2	11.5 ± 0.1	11.2 ± 0.1	4.6 ± 0.0
Deep purple varieties						
Tempranilla negra	73.5 ± 0.4	12.6 ± 0.1	9.5 ± 0.0	14.7 ± 1.0	6.2 ± 0.2	3.9 ± 0.0
Yersey Acriollada	119.2 ± 0.3	16.3 ± 0.6	14.6 ± 0.0	16.5 ± 0.1	10.0 ± 0.1	5.0 ± 0.0
Negra Quiviquinta	118.0 ± 1.1	22.0 ± 0.1	16.4 ± 0.2	17.9 ± 0.1	9.4 ± 0.1	6.4 ± 0.0
China	102.6 ± 0.8	14.7 ± 0.6	13.7 ± 0.3	18.3 ± 0.0	8.5 ± 0.0	4.7 ± 0.0
Morada X Roja	109.8 ± 1.8	17.8 ± 0.9	15.2 ± 0.8	15.1 ± 0.0	7.4 ± 0.0	5.3 ± 0.0
UAN 21	147.4 ± 0.2	21.6 ± 0.8	21.3 ± 0.6	18.0 ± 1.0	13.1 ± 0.2	5.6 ± 0.1
UAN 17	137.6 ± 2.2	23.7 ± 0.1	18.2 ± 0.4	21.0 ± 0.2	12.2 ± 0.0	6.4 ± 0.0
UAN 12_1	154.1 ± 0.4	23.4 ± 0.1	18.5 ± 0.0	22.1 ± 0.0	13.3 ± 0.0	6.4 ± 0.0
UAN 18	100.9 ± 1.6	19.0 ± 0.3	11.6 ± 0.2	13.2 ± 0.3	10.7 ± 0.0	4.5 ± 0.0
UAN 21_1	60.1 ± 2.2	10.0 ± 0.2	8.7 ± 0.2	8.2 ± 0.4	3.4 ± 0.0	2.9 ± 0.2
$6Q_6$	105.5 ± 0.8	15.6 ± 0.1	10.4 ± 0.2	6.9 ± 0.3	9.1 ± 0.0	5.4 ± 0.0
Cruza negra	141.5 ± 0.4	23.1 ± 0.1	19.7 ± 0.6	17.2 ± 0.5	14.9 ± 0.2	7.7 ± 0.1

calyces. The difference between the results reported previously and those of this work could be due to the extraction technique in these: Salinas-Moreno et al. conducted extraction at 92°C for 15 min, a longer time than that of this investigation. It was reported that AA is thermolabile, that its diminution depends on diverse factors (Munyaka et al., 2010), and that in dark hibiscus varieties, AA reduction is greater than in lighter toned varieties. However, a more recent investigation reported that green roselle varieties present a very much lower content of AA than the red varieties (Ademiluyi and Oboh, 2013).

Analysis of the correlation between the antioxidant activity of the aqueous extracts and their concentration of bioactive compounds indicates that there is a positive correlation between their concentration and antioxidant activity, determined by any of the three techniques utilized, even though the correlation coefficients among themselves are lower than those previously reported. Results of antioxidant activity according to the content of phenolic compounds are illustrated in Figure 1.

Anokwuru et al. (2011) reported that the content of TPC is strongly correlated (r = 0.969) with antiradical scavenging capacity in DPPH•, a much greater correlation than that found in the present investigation (r = 0.649; p <0.05). Juliani et al. (2009) reported that between the TPC content and the antioxidant activity of roselle extracts, expressed as antiradical scavenging activity toward the $ABTS^{\bullet +}$ cation, and its anthocyanin coefficients of 0.74 and 0.39, respectively. Prenesti et al. (2007) reported that the phenolic compound content is found to be strongly related with the Briggs-Rauscher Antioxidant Index (BRAI), without indicating the correlation coefficient between these. The authors suggest that it is reasonable to suppose that the antioxidant power of roselle calyx infusions is related exclusively with their content of phenolic compounds. In this investigation, we found that the phenolic compound concentration possessed the lowest correlation with the extracts' antioxidant activity, while AA is that which exhibited the greatest correlation coefficients. The difference between the results of the investigations cited could be attributed to the amount of roselle varieties analyzed; while Prenesti et al. (2007) and Anokwuru et al. (2011) experimented with a sole variety of calyces, this investigation experimented with 64 varieties, which presents a high coefficient of variation in the concentration of bioactive compounds as well as in antioxidant activity.

Antiradical scavenging activity to the DPPH• and total

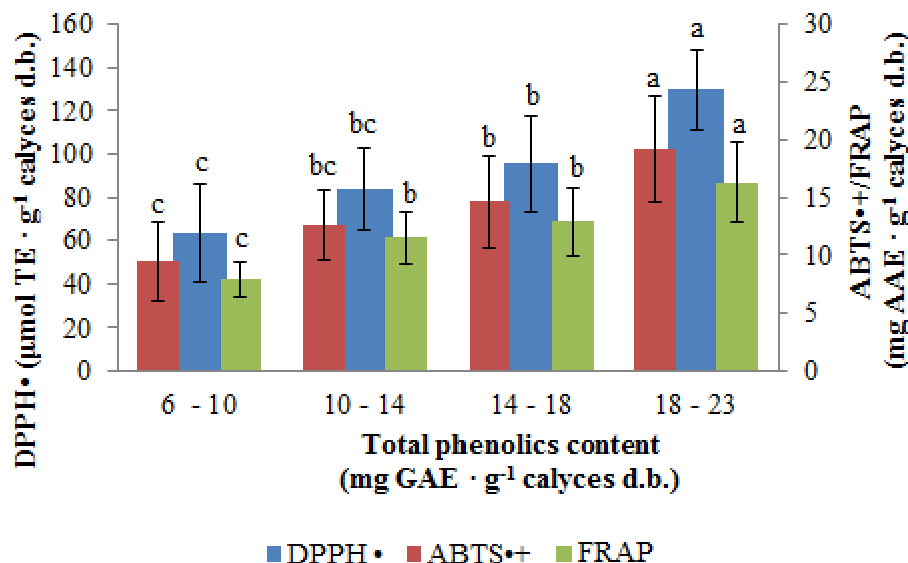

Figure 1. Antioxidant activity according to the total phenolic compound concentration of the 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

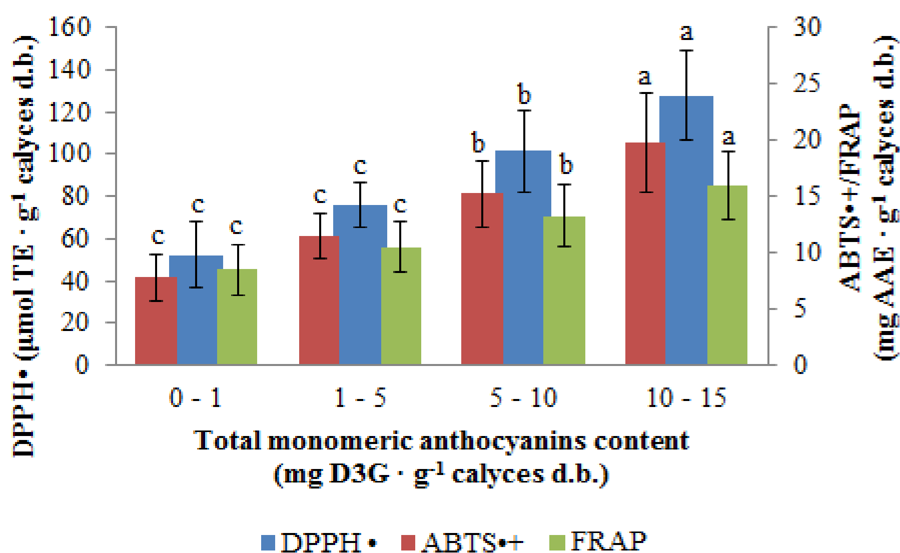

Figure 2. Antioxidant activity according to the total monomeric anthocyanin (TMA) concentration of 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

phenolic concentration had a Pearson *r* correlation coefficient = 0.649 for the scavenging capacity of the ABTS•$^+$ cation: Coefficient *r* was 0.635, and for reductor activity, $r = 0.625$.

Figure 2 shows the values for determination of antioxidant activity according to total monomeric anthocyanin concentration. Correlation coefficients among the determinations were 0.867, 0.829, and 0.745 for the scavenging capacity of the DPPH• free radical, ABTS•$^+$ cation scavenging capacity, and reductor capacity, respectively.

Figure 3 shows the results of the determination of antioxidant activity according to the Ascorbic acid (AA) concentration. Correlation coefficients were 0.891, 0.827, and 0.763 for the 1-1-Diphenyl-2-picrylhydrazyl (DPPH•), 2-2'Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS•$^+$), and Ferric ion reducing antioxidant power (FRAP) assays, respectively.

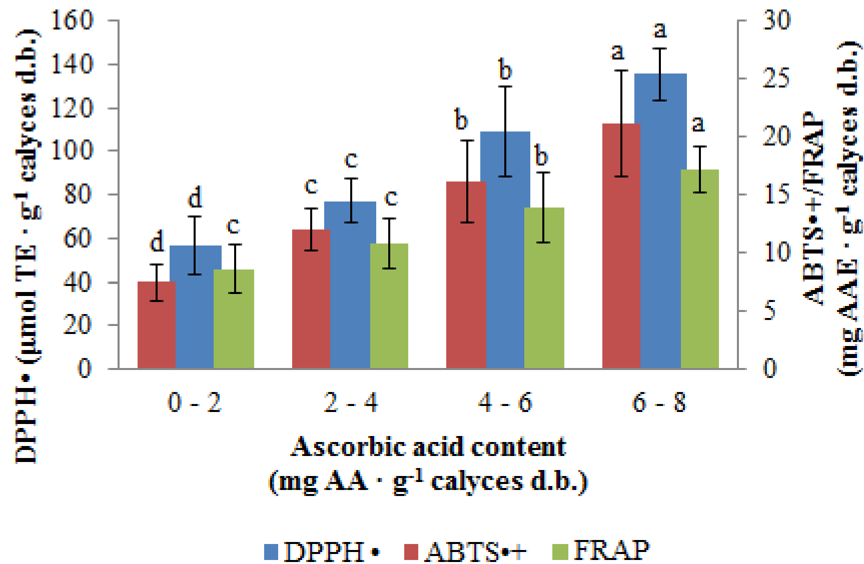

Figure 3. Antioxidant activity according to the ascorbic acid (AA) concentration of 64 varieties of roselle (*jamaica*). Measurements without a letter in common are significantly different according to the Tukey test ($p < 0.05$).

We performed a regression analysis to establish the relationship between the concentration of bioactive compounds analyzed and the antioxidant activity of the aqueous extracts of the 64 varieties of roselle (*jamaica*) subjected to study.

For the scavenging capacity of the DPPH• free radical, the regression equation was Equation (1):

$$DPPH\bullet = 19.4 + 1.62\ TPC + 2.62\ TMA + 8.6\ AA \quad (1)$$

The determination coefficient indicates that 83.9% of the capacity of the roselle aqueous extracts is explained from the concentration of Total phenolic compounds (TPC), or total monomeric anthocyanins (TMA), and of ascorbic acid (AA).

The scavenging capacity of the $ABTS\bullet^{+}$ cation of the aqueous extracts was defined as in Equation (2):

$$ABTS\bullet^{+} = 2.71 + 0.314\ TPC + 0.582\ TMA + 0.831 AA \quad (2)$$

This equation determines that 75.6% of the capacity of the aqueous extracts of the roselle (*jamaica*) varieties subjected to study is due to the concentration of total phenolic compounds (TPC), total monomeric anthocyanins (TMC), and ascorbic acid (AA).

The reductor capacity of the Fe(III) ion into Fe(II) was found to be determined by the Equation (3):

$$FRAP = 3.68 + 0.276\ TPC + 0.298\ TMA + 0.739\ AA \quad (3)$$

64.7% of the reducing capacity of the aqueous extracts of the roselle (*jamaica*) varieties analyzed is due to the concentration of total phenolic compounds (TPC), total monomeric anthocyanins (TMA) and ascorbic acid (AA).

Conclusions

Aqueous extracts of the roselle varieties analyzed present bioactive compound concentrations that confer upon these extracts the capacity of acting as antioxidants. Varieties of roselle with darkly pigmented calyces possess a greater capacity for free radical scavenging as well as for reducing oxidant molecules than light-toned roselle varieties. Due to its greater correlation coefficient, the concentration of ascorbic acid is a better predictor of the antioxidant activity of the aqueous extracts of the roselle varieties analyzed.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Ademiluyi AO, Oboh G (2013). Aqueous extracts of Roselle (*Hibiscus sabdariffa* Linn.) varieties inhibit α-amylase and α-glucosidase activities in vitro. J. Medicinal Food. 16:88–93. http://dx.doi.org/10.1089/jmf.2012.0004

Anokwuru CP, Esiaba I, Ajibaye O, Adesuyi AO (2011). Polyphenolic content and antioxidant activity of *Hibiscus sabdariffa* calyx. Res. J. Medicinal Plant. 5:557–566. http://dx.doi.org/10.3923/rjmp.2011.557.566

Babalola SO, Babalola AO, Aworh OC (2001). Compositional attributes of calyces of roselle (*Hibiscus sabdariffa* L.). J. Food Technol. Afr. 6:133-134. http://www.bioline.org.br/request?ft01035

Christian KR, Jackson JC (2009). Changes in total phenolic and monomeric anthocyanin composition and antioxidant activity of three varieties of sorrel (*Hibiscus sabdariffa*) during maturity. J. Food Compost. Anal. 22:663–667. http://dx.doi.org/10.1016/j.jfca.2009.05.007

Dürüst N,Sümengen D, Dürust Y (1997). Ascorbic acid and element contents of Trabzon (Turkey). J. Agric. Food Chem. 45:2085–2087. http://dx.doi.org/10.1021/jf9606159

Giusti R, Wrolstad RE (2001). Characterization and measurement of anthocyanins by UV-Visible spectroscopy. Curr. Protoc. Food Anal. Chem. pp. 19-31. http://dx.doi.org/10.1002/0471142913.faf0102s00

Gregoris E, Pereira-Lima GP, Fabris S, Bertelle M, Sicari M, Stevanato R (2013). Antioxidant properties of Brazilian tropical fruits by correlation between different assays. BioMed. Res. Int. pp. 1-8. http://dx.doi.org/10.1155/2013/132759

Hinneburg I, Damien-Dorman HJ, Hiltunen R (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 97:122–129. http://dx.doi.org/10.1016/j.foodchem.2005.03.028

Hopkins AL, Lamm MG, Funk JL, Ritenbaugh C (2013). *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. Fitoterapia. 85:84–94. http://dx.doi.org/10.1016/j.fitote.2013.01.003

Juliani HR, Welch CR, Wu Q, Diouf B, Malainy D, Simon JE (2009). Chemistry and quality of Hibiscus (*Hibiscus sabdariffa*) for developing the natural-product industry in Senegal. J. Food Sci. 74:S113–S121. http://dx.doi.org/10.1111/j.1750-3841.2009.01076.x

Khaghani S (2011). Selective cytotoxicity and apoptogenic activity of *Hibiscus sabdariffa* aqueous extract against MCF-7 human breast cancer cell line. J. Cancer Ther. 2:394–400. http://dx.doi.org/10.4236/jct.2011.23054

Kuskoski EM, Asuero AG, Troncoso AM, Mancini-Filho J, Fett R (2005). Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. Ciênc. Tecnol. Alimentos 25:726–732. http://dx.doi.org/10.1590/S0101-20612005000400016

Lin HH, Chan KC, Sheu JY, Hsuan SW, Wang CJ, Chen JH (2012). *Hibiscus sabdariffa* leaf induces apoptosis of human prostate cancer cells in vitro and in vivo. Food Chem. 132:880–891. http://dx.doi.org/10.1016/j.foodchem.2011.11.057

Maganha EG, Halmenschlager RDC, Rosa RM, Henriques JAP, Ramos ALLDP, Saffi J (2010). Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. Food Chem. 118:1–10. http://dx.doi.org/10.1016/j.foodchem.2009.04.005

Morales FJ, Jiménez-Pérez S (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. Food Chem. 72:119–125. http://dx.doi.org/10.1016/S0308-8146(00)00239-9

Mungole A, Chaturvedi A (2011). Determination of antioxidant activity of *Hibiscus sabdariffa* L. and Rumex nepalensis spreng. Int. J. Pharm. Bio Sc. 2:120–128. http://ijpbs.net/volume2/issue1/biological/_19.pdf

Munyaka WA, Indrawati O, Loey AV, Hendrickx M (2010). Application of thermal inactivation of enzymes during vitamin C analysis to study the influence of acidification, crushing and blanching on vitamin C stability in broccoli (*Brassica oleracea* L. var. Italica). Food Chem. 120:591-598. http://dx.doi.org/10.1016/j.foodchem.2009.10.029

Prenesti E, Berto S, Daniele PG, Toso S (2007). Antioxidant power quantification of decoction and cold infusions of *Hibiscus sabdariffa* flowers. Food Chem. 100:433–438. http://dx.doi.org/10.1016/j.foodchem.2005.09.063

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231–1237. http://dx.doi.org/10.1016/S0891-5849(98)00315-3

Salinas-Moreno Y, Zúñiga-Hernández ARE, Jiménez-De la Torre LB, Serrano-Altamirano V, Sánchez-Feria C (2012). Color en cálices de jamaica (*Hibiscus sabdariffa* L.) y su relación con características fisicoquímicas de sus extractos acuosos. Rev. Chapingo Serie Hortic. 18:395-407. http://dx.doi.org/10.5154/r.rchsh.2011.08.038

Stintzing FC, Herbach KM, Mosshammer MR, Carle R, Yi W, Sellappan S (2005). Color, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. J. Agric. Food Chem. 53:442–451. http://dx.doi.org/10.1021/jf048751y

Tsai PJ, McIntosh J, Pearce P, Camden B, Jordan BR (2002). Anthocyanin and antioxidant capacity in roselle (*Hibiscus sabdariffa* L.) extract. Food Res. Int. 35:351–356. http://dx.doi.org/10.1016/S0963-9969(01)00129-6

Yin M, Chao C (2008). Anti-Campylobacter, anti-aerobic and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. Int. J. Food Microbiol. 127:73–77. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.06.002

Filename: Medina-Carrillo et al pdf
Directory: \\192.168.1.24\qa operation data\Agric. Sci\AJAR\2015
Template: C:\Users\Daniel\AppData\Roaming\Microsoft\Templates\Normal.dotm
Title: Nutrients
Subject:
Author: Daniel
Keywords:
Comments:
Creation Date: 3/14/2015 10:51:00 AM
Change Number: 20
Last Saved On: 4/15/2015 2:50:00 PM
Last Saved By: Daniel
Total Editing Time: 54 Minutes
Last Printed On: 4/15/2015 2:50:00 PM
As of Last Complete Printing
Number of Pages: 8
Number of Words: 4,877 (approx.)
Number of Characters: 27,805 (approx.)