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# Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*)

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ABSTRACT

This study reports the phytochemical profile (phenolics, flavonoids and anthocyanins), the antioxidant capacity and the antibacterial activity of the ethanolic extracts of a collection of 25 Mexican *Hibiscus sabdariffa* (Hs) varieties with different calyx color intensities, from green–yellow to deep red, cultivated in the same condition. A great variety of phenolic compounds were identified in the different extracts using HPLC-DAD-ESI-TOF-MS, mainly phenolic acids, flavonoids, and anthocyanidin, some of which have been described for the first time. The total phenolic, flavonoid and anthocyanidin contents showed great variation in the different Hs extracts, ranging from 2400 ± 300 to 10,000 ± 400 mg gallic acid equivalents (GAE)/100 g dry calyx (dc), from 419 ± 2 to 2260 ± 70 mg quercetin/100 g dc, and from 0 to 4408 mg/100 g dc with different ratios of delphinidin:cyanidin, respectively. Furthermore, the antioxidant capacity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay presented the same behavior, with values that varied from 27.4 ± 0.3 to 112 ± 8 mol equivalent trolox/g dc. In addition, the antibacterial activity of the HS extracts was assayed against Gram-negative (*Escherichia coli*, *Salmonella enteritidis*) and Gram-positive (*Staphylococcus aureus*, *Micrococcus luteus*) microorganisms, demonstrating that the ethanol extracts were effective against all the bacterial strains tested, showing a greater effect against Gram-positive bacteria. Finally, a multivariate analysis for the classification of the Hs varieties has been carried out based on the anthocyanidin content. The results reported in this paper shows for the first time antibacterial and phytochemistry diversity of the different varieties of Hs, which highlights the importance of a correct description of Hs materials used in research papers.



## 1. Introduction

*Hibiscus sabdariffa* L. (Hs; Malvaceae), commonly known as roselle, red sorrel or karkade, is an annual herbaceous subshrub belonging to the family Malvaceae. This plant is native to Africa and grows in tropical and sub-tropical regions such as Sudan, southern Asia, and America. Fleshy calyces (sepals) are commercially important for the production of beverages, juices, jams, and syrup in the food industry. Furthermore, these calyces are a good source of natural food colorants because of their high pigment content (Bridle and Timberlake, 1997). Moreover, the dried calyces are consumed worldwide in hot infusions and in cold drinks (Hervert-Hernández and Goñi, 2012). Besides its extended consumption as a beverage and its uses in food industry, roselle is also used in animal feed, nutraceuticals, cosmetics and pharmaceuticals (Wang et al., 2012).

Hs plant contains proteins, fats, carbohydrates, acids, minerals, vitamins, as well as many types of phenolic compounds. Mucilaginous polysaccharides and pectins also form part of its composition. Furthermore, Hs seeds contain a wide range of less polar compounds, such as sterols (sitosterol, ergosterol, campesterol). Although the composition of this plant has been thoroughly

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studied, one notable (and underreported) group of compounds present in this plant is phenolic compounds, which recently have attracted a great deal of attention due to their beneficial effects in the promotion of human health and well-being (Ali et al., 2005; Crozier et al., 2009; Rodríguez-Medina et al., 2009).

The phenolic compounds found in this plant include organic and phenolic acids, such as citric acid, hydroxycitric acid, hibiscus acid, and protocatechuic acid. Flavonoids such as quercetin, luteolin or gossipetin, and their respective glycosides are also present. Anthocyanins, detected in high amounts in the calyces, are responsible for the bright red color. The most frequent anthocyanins of Hs flowers are cyanidin-3-glucoside, delphinidin-3-glucoside, cyanidin-3-sambubioside, and delphinidin-3-sambubioside (Ali et al., 2005; Rodríguez-Medina et al., 2009).

Numerous pharmacological properties have been attributed to Hs calyx (Ali et al., 2005). The most significant ones are its cardioprotective action, antihypertensive action, effectiveness against low-density lipoprotein oxidation, and hyperlipidemia (Alarcón-Aguilar et al., 2007; Beltrán-Debón et al., 2010; Gurrola-Díaz et al., 2010; Fernández-Arroyo et al., 2012; Herranz-López et al., 2012; Kuo et al., 2012; Laikangbam and Damayanti Devi, 2012; Hopkins et al., 2013). Also potent effects in the reduction of urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium, and phosphate have been demonstrated (Kuo et al., 2012; Laikangbam and Damayanti Devi, 2012). Moreover, its anti-inflammatory, antioxidative, hepatoprotective, and antitumoral effects have been highlighted (Fernández-Arroyo et al., 2011; Lin et al., 2012; Wang et al., 2000).

Most of the research on Hs does not specify the origin of the variety and the crop site, making it difficult to make comparisons between the phytochemical profile and biological properties of extracts obtained in different studies due to the inherent variability of the variety and harvesting location of a crop. The aim of the present work has been to identify the phenolic composition by HPLC-DAD-ESI-TOF-MS of the ethanolic extract in a collection of 25 different varieties of Hs from Mexico and determining the content of phenolic, flavonoid, antocyanidin and antioxidant activity as well as their antibacterial activity against *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Micrococcus luteus*.

## 2. Material and methods

### 2.1. Chemicals

All chemicals used were of analytical HPLC reagent grade. Formic acid, trifluoroacetic acid, methanol and acetonitrile, used for preparing mobile phases, were purchased from Fluka, Sigma–Aldrich (Steinheim, Germany) and Lab-Scan (Gliwice, Sowinskiego, Poland), respectively. Water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). The standards, for the comparison with the compounds detected, chlorogenic acid, cyanidin chloride, delphinidin chloride, quercetin, and quercetin-3-glucoside were purchased from Fluka, Sigma–Aldrich (Steinheim, Germany) and Extrasynthese (Genay Cedex, France). The stock solutions containing these analytes were prepared in methanol (Lab-Scan), and the hydrochloric acid (HCl) used for anthocyanin determination was purchased from Lab-Scan (Gliwice, Sowinskiego, Poland).

### 2.2. Sample collection and processing

Seeds of 25 different varieties of Hs were collected from the main producing areas in Mexico during the years 2007–2010 (Table 1). The different varieties were established in the field under the same conditions until physiological maturity and then the calyces were collected and dried in a forced convection hot-air oven. A total of 100 g of dried calyxes of each Hs variety were grounded and extracted using acidified ethanol (70% ethanol plus 0.1% HCl) with a solvent to sample extract ratio of 1:10 (w/v). After 72 h of extraction, solvent was evaporated at 40 °C using a rotary evaporator (Büchi R-215, Büchi Labortechnik AG, Switzerland) under high

**Table 1**
Content of total phenolic compound, flavonoids, anthocyanin, delphinidin, cyanidin and antioxidant capacity in *H. sabdariffa* extracts.

| HS extract | Variety | Origin | Total phenolic content (mg GAE/g dc) | Flavonoid content (mg QE/ 100 g dc) | Anthocyanidin content** (mg/100 g dc) | Delphinidin content (mg/100 g dc) | Cyanidin content (mg/100 g dc) | Antioxidant capacity (μmol ET/ g dc) |
|---|---|---|---|---|---|---|---|---|
| HS 1 | Americana | Jalisco | $30 \pm 6^{hi}$ | $813 \pm 5^{i}$ | $969^{m}$ | $444^{k}$ | $525^{h}$ | $104.0 \pm 9^{a}$ |
| HS 2 | Tepalcatepec | Michoacán | $90 \pm 20^{b}$ | $692 \pm 8^{jk}$ | $1673^{i}$ | $1083^{g}$ | $589^{f}$ | $83.0 \pm 0.6^{dc}$ |
| HS 3 | Diamante | Jalisco | $98 \pm 1^{a}$ | $1695 \pm 8^{b}$ | $2967^{d}$ | $2232^{c}$ | $734^{c}$ | $112.0 \pm 8^{a}$ |
| HS 4 | Colima | Colima | $94 \pm 9^{b}$ | $1310 \pm 40^{d}$ | $2306^{f}$ | $1658^{e}$ | $648^{d}$ | $60.1 \pm 1^{fe}$ |
| HS 5 | Tempranilla | Jalisco | $41 \pm 3^{g}$ | $429 \pm 5^{n}$ | $4408^{a}$ | $3535^{a}$ | $873^{a}$ | $40.0 \pm 2^{ji}$ |
| HS 6 | Talpa | Jalisco | $38 \pm 5^{g}$ | $419 \pm 2^{n}$ | $4085^{c}$ | $3300^{b}$ | $784^{b}$ | $45.0 \pm 8^{i}$ |
| HS 7 | Violenta | Jalisco | $60 \pm 10^{e}$ | $720 \pm 30^{j}$ | $4246^{b}$ | $3495^{a}$ | $750^{c}$ | $48.6 \pm 0.4^{i}$ |
| HS 8 | Quesería | Colima | $57 \pm 9^{ef}$ | $530 \pm 20^{l}$ | $1781^{h}$ | $1223^{f}$ | $558^{g}$ | $105.8 \pm 0.3^{a}$ |
| HS 9 | CriollaTala | Jalisco | $64 \pm 2^{e}$ | $890 \pm 10^{hi}$ | $1369^{j}$ | $825^{h}$ | $543^{g}$ | $80.0 \pm 2^{dc}$ |
| HS 10 | Tecoman | Colima | $42 \pm 4^{g}$ | $470 \pm 10^{n}$ | $1013^{m}$ | $511^{k}$ | $501^{i}$ | $37.0 \pm 1^{k}$ |
| HS 11 | El Bordo | Colima | $34 \pm 3^{h}$ | $1030 \pm 70^{gh}$ | $1308^{k}$ | $783^{h}$ | $525^{h}$ | $76.6 \pm 0.3^{ed}$ |
| HS 12 | Tempranilla | Colima | $45 \pm 9^{fg}$ | $1140 \pm 50^{fe}$ | $2613^{e}$ | $1956^{d}$ | $657^{d}$ | $84.0 \pm 2^{bcd}$ |
| HS 13 | Sudan | Colima | $43 \pm 4^{g}$ | $488.0 \pm 0.6^{m}$ | $929^{m}$ | $436^{k}$ | $492^{i}$ | $36.3 \pm 0.3^{k}$ |
| HS 14 | M. Luna | Colima | $70 \pm 10^{d}$ | $681 \pm 7^{k}$ | $1500^{j}$ | $940^{h}$ | $560^{g}$ | $38.5 \pm 0.1^{j}$ |
| HS 15 | Pisila | Colima | $68 \pm 3^{d}$ | $740 \pm 20^{ji}$ | $1361^{k}$ | $791^{h}$ | $569^{g}$ | $56.0 \pm 2^{fgh}$ |
| HS 16 | JB 01 SM | Guerrero | $83 \pm 1^{c}$ | $1050 \pm 30^{g}$ | $0^{n}$ | $0^{l}$ | $0^{j}$ | $84.0 \pm 8^{bcd}$ |
| HS 17 | JCP 01T | Guerrero | $88.8 \pm 0.6^{cb}$ | $950 \pm 30^{h}$ | $1058^{L}$ | $552^{j}$ | $505^{i}$ | $56.0 \pm 3^{fg}$ |
| HS 18 | Blanca | Guerrero | $81 \pm 1^{c}$ | $1272 \pm 10$ | $0^{n}$ | $0^{l}$ | $0^{j}$ | $49.2 \pm 0^{i}$ |
| HS 19 | JJ 01 SM | Guerrero | $83 \pm 1^{c}$ | $810 \pm 50^{i}$ | $1216^{L}$ | $663^{i}$ | $553^{g}$ | $48.6 \pm 0.5^{i}$ |
| HS 20 | JR 01 C | Guerrero | $37 \pm 5^{gh}$ | $1070 \pm 10^{fg}$ | $1147^{l}$ | $633^{i}$ | $514^{h}$ | $85.0 \pm 0.9^{bcd}$ |
| HS 21 | Americana | Colima | $51 \pm 7^{f}$ | $504 \pm 4^{m}$ | $1815^{h}$ | $1205^{f}$ | $609^{e}$ | $35.1 \pm 0.1^{kl}$ |
| HS 22 | P. Anzar | Colima | $28 \pm 4^{i}$ | $632 \pm 5^{kl}$ | $1178^{l}$ | $678^{i}$ | $500^{i}$ | $97.3 \pm 8^{bc}$ |
| HS 23 | Blanca | Jalisco | $24 \pm 3^{j}$ | $855 \pm 8^{hi}$ | $0^{n}$ | $0^{l}$ | $0^{j}$ | $27.4 \pm 0.3^{l}$ |
| HS 24 | Real | Veracruz | $100 \pm 4^{a}$ | $1500 \pm 70^{c}$ | $1280^{k}$ | $707^{i}$ | $572^{f}$ | $101.0 \pm 6^{b}$ |
| HS 25 | Reyna | Nayarit | $70 \pm 1^{d}$ | $2260 \pm 70^{a}$ | ND | ND | ND | $97.0 \pm 4^{bc}$ |

*Mean values in a column superscripted by the same letter are not significantly different at $p < 0.05$.
ND: Not determined.
** Anthocyanidin content = Delphinidin + Cyanidin.