Dietary Fiber Content and Associated Antioxidant Compounds in Roselle Flower 
(*Hibiscus sabdariffa* L.) Beverage

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The beverage of *Hibiscus sabdariffa* flowers is widely consumed in Mexico. Polyphenols contained in plant foods are frequently associated with dietary fiber. The aim of this work is to quantify the dietary fiber, associated polyphenols, and antioxidant capacity of the Roselle flower and the beverage traditionally prepared from it and its contribution to the Mexican diet. Roselle flower contained dietary fiber as the largest component (33.9%) and was rich in phenolic compounds (6.13%). Soluble dietary fiber was 0.66 g/L in beverage, and 66% of total extractable polyphenols contained in Roselle flower passed to the beverage and showed an antioxidant capacity of 335 µmol trolox equivalents/100 mL beverage measured by ABTS. These data suggest that Roselle flower beverage intake in the Mexican diet may contribute around 166 and 165 mg/per serving to the intake of dietary fiber and polyphenols, respectively. The health benefits from consumption of *Hibiscus* beverage could be of considerable benefit to the whole population.

**KEYWORDS:** *Hibiscus sabdariffa*; soluble dietary fiber; polyphenols; antioxidant capacity

**INTRODUCTION**

Today, plants with dietary fiber (DF) and bioactive compounds are of growing interest to researchers because of their linkage to human health. *Hibiscus sabdariffa* L. (family Malvaceae), commonly known as roselle, red sorrel, or karkadé, is widely grown in Africa, South East Asia, and some tropical countries of America (1). The fleshy flowers provide a soft drink consumed as a cold or hot beverage (2, 3). The daily consumption of this beverage, called “flor de jamaica” in Mexico (4) and “sobo” in Nigeria (1), is high because of the sensation of freshness conveyed.

Pharmacological actions have been identified in *H. sabdariffa* flowers, petals, and seeds (3). The healthy effects are numerous: cardioprotective action (5, 6); reduction of urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium, phosphate (7); antihypertensive action (4, 8); effectiveness against low-density lipoprotein oxidation and hyperlipidemia (6).

Roselle is an important source of vitamins, minerals, and bioactive compounds, such as organic acids, phytosterols, and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitosterol and ergosterol (3).

Polyphenols contained in plant foods are frequently associated with DF (9). Both polyphenols and DF can be released from the food matrix during preparation of the beverage. Various authors (2, 10) have evaluated phenolic content and the antioxidant activity in this material. However, to our knowledge none of the works address the possibility that the beverage may contain DF with associated polyphenols and antioxidant properties. It is also worth noting that the daily intake of large amounts of these beverages in some countries like Mexico may constitute an important source of natural antioxidants for whole population, including children, elderly, and other risk groups.

Some researchers have focused on Roselle water extracts (11, 12), while others have employed an organic solvent to extract possible bioactive compounds (13). Indeed, the different extraction techniques complicate comparisons among studies. Moreover, different varieties of *Hibiscus sabdariffa* have been analyzed, and as far as we know, little has been published regarding the composition of Mexican *Hibiscus sabdariffa* and the nutritional features of the beverage.

The aim of this work was to quantify the fiber content in the flower and the beverage obtained from *H. sabdariffa*, the associated polyphenol compounds, and the antioxidant capacity. It also includes an estimation of the contribution of this beverage
to the daily intake of dietary fiber, polyphenols, and antioxidant capacity in the Mexican diet.

MATERIALS AND METHODS

**Roselle Flower.** Sample Preparation. *Hibiscus sabdariffa* L., flowers packed bags (Mydac, S.A. de C.V. Central de Abasto, México), was acquired from a local supermarket in Acapulco, México. A portion of the sample was freeze-dried and sieved in mesh (≤0.5 mm) and kept in a container.

**Analytical Determination.** Crude protein was determined in a pure oxygen environment on a furnace. After passing the sample through a thermostatic cooler to remove water, an aliquot of the combustion gases was taken. Gases were bubbled, and all the nitrogen-containing materials were reduced to nitrogen and detected by a thermal conductivity cell. An air blank was used, and the instrument was calibrated with EDTA. Protein was calculated as nitrogen × 6.25.

**Dietary Fiber.** It was analyzed by the AOAC enzymatic–gravimetric method (15) using protease, heat-stable α-amylase, and amyloglucosidase to remove protein and starch. Remaining residues were separated by centrifugation (15 min, 25 °C, 3000g), and the supernatants were dialyzed to avoid losses of soluble dietary fiber (SDF), as reported by Mañas and Saura-Calixto (16). Uronic acids (UA) and neutral sugars (NS) were determined spectrophotometrically at 520 nm by the Englyst and Cummings (17) method, and glucose was used as a standard. Insoluble dietary fiber (IDF) was quantified gravimetrically. Ash content was determined in triplicate in an electric furnace for 16 h at 550 °C quantified gravimetrically.

**Extractable Polyphenols (EPP).** The sample was extracted by shaking at room temperature with methanol–water (50:50 v/v, 50 mL/g sample, 60 min, constant shaking) and acetone–water (70:30 v/v, 50 mL/g sample, 60 min, constant shaking). After centrifugation (15 min, 25 °C, 3000g) supernatants were combined, and total polyphenols were determined by the Folin–Ciocalteau procedure (18). The main groups of phenolic compounds were identified by HPLC following the method described by Lamuela-Raventos and Waterhouse (19). A Hewlett-Packard 1100 liquid chromatograph with a diode array detector couple to a Chemstation HP 79995 was used. A Novapack column C-18 (250 mm × 4 mm), 5 μm particle size, from Waters/Millipore was used for the stationary phase with a flow of 0.5 mL/min. The volume injected was 100 μL. The solvents used for the separation were as follows: solvent A = 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B = 20% A; solvent C = 80% acetonitrile; and solvent C = 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5. Quantification was made at 280 nm for benzoic acids (expressed as gallic acid) and for flavan-3-ols (expressed as catechin), at 320 nm for hydroxyxinnamic acids (expressed as caffeic acid), at 365 nm for flavonols (expressed as rutin), and at 520 nm for anthocyanidins (expressed as malvidin).

**Nonextractable Polyphenols (NEPP).** Proanthocyanidins were determined in the residue of the methanol/acetone/water extraction. The residue was treated with 5 mL/L HCl–butanol (3 h, 100 °C) (20) for proanthocyanidins hydrolysis. Proanthocyanidins were calculated from the absorbance at 550 nm of an anthocyanidin solution from Mediter-

**Antioxidant Capacity Assay.** Supernatants extracted from flowers of Roselle described before were employed to estimate the antioxidant capacity content by FRAP, ABTS, and ORAC assays.

**Ferric Reducing Ability Assay (FRAP).** The method was described by Benzie and Strain (22). Briefly, FRAP reagent, containing 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (Fluka Chemicals, Madrid, Spain), FeCl3, and acetate buffer, was mixed with 90 μL of distilled water and 30 μL of the test sample or the blank (solvents used for extraction). Absorbance values at 595 nm were taken every 15 s at 37 °C, using a Beckman DU-640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). The readings at 30 min were selected for calculations of FRAP values. A standard curve of trolox was used to estimate antioxidant capacity of samples. It was expressed as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble analogue of vitamin E) equivalents.

**Free Radical Scavenging Assay (ABTS).** The antioxidant capacity was estimated in terms of radical scavenging activity following the procedure described elsewhere (23) with some modification (24). Briefly, ABTS radical cation (ABTS⁺) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate in the dark at room temperature for 12–16 h before use. The ABTS⁺ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 730 nm. After addition of 0.1 mL of sample to 3.9 mL of diluted ABTS⁺ solution, absorbance readings were taken every 20 s using a Beckman DU-640 spectrophotometer. The reaction was monitored for 6 min. Inhibition of absorbance vs time was plotted, and the area below the curve (0–6 min) was calculated. Solutions of known trolox concentration were used as antioxidant capacity equivalents.

**Oxygen Absorbance Radical Capacity (ORAC) Assay.** The procedure described by Ou et al. (25) slightly modified was as follows: 175 μL of the sample/blank was mixed with 120 μL of phosphate buffer saline (PBS) 75 (mM), pH 7.4, 205 μL of a 2,2′-azobis(2-amidinopropane) dichloride solution 53 (mM), and 3 mL of a fluorescein solution 48 nM. Fluorescence was recorded until it reached zero (excitation wavelength 493 nm, emission wavelength 515 nm) in a fluorescence spectrophotometer (Perkin-Elmer LS 55) equipped with an automatic thermostatic autocell holder at 37 °C. Results were calculated using the differences of areas under the fluorescein decay curve between the blank and the sample and are expressed as trolox equivalents.

**Roselle Flower Beverage.** Sample Preparation. The beverage was prepared following a popular procedure in Mexico. Briefly, 5 g of *Hibiscus sabdariffa* L. flowers was decoced with 100 mL of distilled water for 5 min. The beverage was rapidly filtered through a Buchner funnel with a Whatman No. 4 filter paper and then kept in refrigeration for 24 h until analysis.

**Soluble Dietary Fiber Determination.** Soluble dietary fiber was determined following an enzymatic method recently described by Díaz-Rubio and Saura-Calixto (26). Briefly, 100 mL of brewed Roselle flower was treated with pepsin (pH 1.5, 40 min, 40 °C, 2000 FIP-U/g, Merck 7190), α-amylase (pH 6.9, 3 h, 37 °C, 17.5 U/Mg, Sigma A3176), and amyloglucosidase (pH 4.75, 45 min, 60 °C, 14 U/Mg, Roche 102857). After enzymatic treatments soluble fiber was isolated by dialysis at constant temperature to eliminate all enzymatic hydrolysis products. Samples were transferred into dialysis tubes (12–14 kDa molecular weight cutoff, Dialysis Tubing Visking, Medicell International, London, UK) and dialyzed against water for 48 h at 25 °C (water flow 7 L/h). Quantitative analysis of fiber in the solutions retained in the dialysis tubes was performed. Retentants were hydrolyzed with 1 M sulfuric acid (final acid concentration of the solution) at 100 °C for 90 min, and soluble dietary fiber was measured spectrophotometrically (17) with dinitrosalicylic acid (Panreac 162837, Barcelona, Spain).

**Polyphenols Determination.** Polyphenols were determined directly in Roselle beverage by the Folin–Ciocalteau procedure (18). Gallic acid was used as standard, and the polyphenolic content was expressed as gallic acid equivalents. The main groups of phenolic compounds were identified by HPLC following the Lamuela-Raventos and Waterhouse (19) method described above.

**Polyphenols Associated with Soluble Dietary Fiber.** They were determined in the soluble dietary fiber isolated after the dialysis procedure following the procedure described above by the Folin–Ciocalteau method (18). Gallic acid was used as standard, and results were expressed as gallic acid equivalents.

**Antioxidant Capacity.** It was determined by FRAP, ABTS, and ORAC assays, as described previously.
The composition of the Roselle flower (Hibiscus sabdariffa L.) (Table 1) was similar to referenced data, with some differences that may be due to genetic variety and type of soil (2). Some authors have reported high concentrations of organic acids such as malic, tartaric, and citric acid in the flower, with the last of these predominating (3); these compounds have not been determined in this work. Minerals determined as ash were an important component in the Hibiscus studied, the main mineral components being potassium, calcium, magnesium, and zinc. However, the mineral content was dependent on soil type and plant growth environment (2).

DF was the largest component of the flowers. This fraction was rich in insoluble compounds (85.6%) while soluble dietary fiber (SDF) was 14.4% of the total DF content. As reported by El-Hamidi et al. (27), the petals of H. sabdariffa yielded 65% dry weight of mucilage, which on hydrolysis produced galactose, galacturonic acid, and rhamnose. Three water-soluble polysaccharides composed of arabinans and arabinogalactans of low molecular mass phenolics that can be extracted using different organic and organic–aqueous solvents (e.g., water, methanol, and aqueous acetone); NEPPs are high molecular mass compounds (proanthocyanidins and hydrolyzable phenolics) or polyphenols bound to other food matrix components such as DF and protein that can be found in the residues of aqueous–organic extracts. Most of the referenced data correspond to polyphenols analyzed in aqueous–organic extracts of foods (EPP), while a significant amount of potentially bioactive polyphenols that remain in the residues (NEPP) is usually ignored (9). Our results indicate that the sample contained both EPPs and NEPPs, the latter being the more abundant (principally proanthocyanidins) (Table 2). The EPP values are similar to those reported by other authors (11–13); however, NEPP data in the literature are scarce. Spectral identification detected four groups of extractable polyphenols: hydroxybenzoic acids, hydroxycinnamates, flavonols, and anthocyanidins, and the corresponding percentage distribution in EPP is shown in Table 2. From a physiological point of view, it is useful to distinguish between soluble or extractable forms of polyphenols and bound or nonextractable forms, which present different bioaccessibility in the gastrointestinal tract. Low molecular mass polyphenols appear to be absorbed from the digestive tract and produce systemic effects (28), while bound and highly polymerized polyphenols are not bioaccessible at all in the small intestine but may be partially degraded by colonic microbiota (9). The bulk of them quantitatively is recovered in feces (29).

### Table 1. Composition of Roselle Flower (Hibiscus sabdariffa L.) g/100 g Dry Mattera

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>9.67 ± 0.28</td>
</tr>
<tr>
<td>Fat</td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td>Ash</td>
<td>9.75 ± 0.59</td>
</tr>
<tr>
<td>Soluble sugars</td>
<td>4.38 ± 0.05</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>33.9 ± 3.56</td>
</tr>
<tr>
<td>Soluble dietary fiber</td>
<td>4.9 ± 0.17</td>
</tr>
<tr>
<td>Insoluble dietary fiber</td>
<td>29.04 ± 3.56</td>
</tr>
</tbody>
</table>

aData are means ± SEM, n ≥ 6. 6 N × 6.25.

### RESULTS AND DISCUSSION

Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of Hibiscus sabdariffa L. are reported in the literature (12). Roselle flower organic extract showed antioxidant capacity (Table 2) that seems to be a consequence of the polyphenol content and other antioxidants such as ascorbic acid (2, 3), eugenol, and limonene (6). The differences in the values produced by FRAP, ABTS, and ORAC, which possibly reflect the influence of different factors on the effectiveness of antioxidants in complex heterogeneous foods, mean that antioxidant capacity cannot be evaluated using only one assay protocol. FRAP method measures total reductive power, while ABTS and ORAC measure free radical scavenging capacity. The antioxidant capacity observed in ORAC was noticeably different from that of the other two assays, probably because of the apolar solvent used in this method (30).

Flowers can also be used as a culinary resource (2, 10); their DF, bioactive compounds, and antioxidant capacity are good reasons to foster their use as a source of antioxidant dietary fiber (9), and they may be suitable for use as an ingredient in functional foods or nutritional supplements.

### Soluble Dietary Fiber Content and Antioxidant Capacity of Roselle Flower Beverage.

The beverage made from Roselle decocction contains compounds of nutritional value such as soluble dietary fiber (SDF) and phenolic compounds. DF content is generally underestimated in analyses of beverages, and food composition tables report zero DF content for most beverages. However, a significant part of SDF contained in original sample (e.g., grape, fresh fruit, roselle) may pass into the beverage (e.g., wine, juices, decoctions) during preparation. A newly developed analytical method to quantify SDF content in beverages was reported recently (26) and was used in this work. Part of the SDF (Table 3) and phenolic compounds contained in Roselle flowers were extracted during the preparation of the beverage.

The Roselle beverage contained 0.66 g of SDF per liter (Table 3). This is low compared with the DF content of solid plant foods, but it is comparable to the contents in other beverages such as beer (0.2 g/L), white wine (0.19 g/L), or red wine (1.37 g/L) (26). In Mexico and other countries the Roselle beverage is drunk as part of a meal or during the day as a cold drink. If we estimate a serving of 250 mL Roselle beverage, the contribution of this beverage to the intake of SDF in Mexico would be around 166 mg/per serving, which represents about 2% of the recommended SDF intake. The contribution to DF intake is similar to that of other beverages such as beer or wine (26). However, it is important to note that Roselle beverage is a nonalcoholic drink consumed by the whole population and may enhance the quality of the diet by increasing the intake of SDF and phenolic compounds with antioxidant activity.

### Table 2. Total Phenolic Content in Roselle Flowers (Hibiscus sabdariffa L.) Dry Mattera

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extractable polyphenols (g GAE/100 g)</td>
<td>2.17 ± 0.04</td>
</tr>
<tr>
<td>Hydroxybenzoic acids (%)</td>
<td>32.6</td>
</tr>
<tr>
<td>Hydroxycinnamic acids (%)</td>
<td>30.6</td>
</tr>
<tr>
<td>Anthocyanidins (%)</td>
<td>30.8</td>
</tr>
<tr>
<td>Flavonols (%)</td>
<td>5.87</td>
</tr>
<tr>
<td>Nonextractable polyphenols (g GAE/100 g)</td>
<td>3.38 ± 0.06</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>66.3 ± 11.2</td>
</tr>
<tr>
<td>Hydrolyzable polyphenols</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Antioxidant capacity (µmol trolox equivalents/g)</td>
<td>90.8 ± 34.8</td>
</tr>
<tr>
<td>FRAP</td>
<td>303.5 ± 8.2</td>
</tr>
</tbody>
</table>

aData are means ± SEM, n ≥ 9.
FRAP and ABTS was similar to that of white wine (35) and lower than that of black tea or orange juice (24). The FRAP assay is based on the reducing ability of antioxidant compounds present in the sample. The high coefficients ($R^2 = 0.8792$) indicate a relatively strong correlation between FRAP and ABTS. The values obtained with the oxygen absorbance radical capacity assay (ORAC) (which shows the free radical scavenging capacity of the polyphenol components of the beverage) were similar to those of red grape juice and higher than those of white grape juice reported by Dávalos et al. (36). The extraction yield in the beverage was lower than in the organic extract (Table 3), and ORAC produced the lowest yield, probably influenced by the solvent employed (water) (33); however, the antioxidant capacity per serving of the beverage was notable.

To summarize, soluble dietary fiber is a quantitatively important constituent of *Hibiscus sabdariffa* L. beverage, and it contributes significantly to the daily intake of SDF in Mexico. Moreover, this SDF contains associated polyphenols which confer antioxidant activity, possibly inducing a healthy effect in the colon. Also, polyphenols bound to the soluble DF can reach the colon, where they may counteract the effects of dietary microflora metabolism, contributing to a healthy status.

### LITERATURE CITED


13. Farombi, E. O.; Fakoya, A. Free radical scavenging and antioxidant activities of natural phenolic compounds in dried flowers.


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