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 karkadè, 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* L. 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-sitoesterol and ergoesterol (*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

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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 part of the sample was freeze-dried and sieved in mesh (≤0.5 mm) and kept in a container.

Analytical Determination. Crude protein was combusted in a pure oxygen environment on a furnace. After passing the sample through a thermoelectric 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. Soluble sugars were quantified by the spectrophotometric anthrone method at 620 nm in 84% v/v sulfuric acid. Glucose was used as a standard (*14*). Fat was determined in triplicate using a Soxhlet system HT extractor for 1 h with petroleum ether and quantified gravimetrically. Ash content was determined in triplicate in an electric furnance for 16 h at 550 °C quantified gravimetrically.

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, 3000*g*), 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 spectrometrically at 520 nm by the Englyst and Cummings (*17*) method, and glucose was used as a standard. Insoluble dietary fiber (IDF) was quantified gravimetrically. The IDF value was expressed as percentage of edible dry matter.

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, 3000*g*) supernatants were combined, and total polyphenols were determinated 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 with 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 hydroxycinnammic 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 solutions from Mediterranean carob pod (*Ceratonia siliqua* L.) supplied by Nestlé S.A. treated under the same conditions to obtain standard curves. Hydrolyzable polyphenols were determined by a methanol/H_2SO_4 90:10 (v/v) hydrolysis at 85 °C for 20 h from the residues of methanol/acetone/water extraction (*21*); after centrifugation (15 min, 25 °C, 3000*g*) supernatants were combined and used to determinate the hydrolyzable polyphenols by the Folin–Ciocalteu method (*18*).

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), $FeCl_3$, 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 ($ABTS^{\bullet+}$) 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 $ABTS^{\bullet+}$ 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 $ABTS^{\bullet+}$ 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 an 2,2′-azobi(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 decocted 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–Ciocalteu 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.

Table 1. Composition of Roselle Flower (*Hibiscus sabdariffa* L.) g/100 g Dry Matter[a]

protein[b]	9.87 ± 0.28
fat	0.59 ± 0.06
ash	9.75 ± 0.59
soluble sugars	4.38 ± 0.05
total dietary fiber	33.9 ± 3.56
soluble dietary fiber	4.9 ± 0.17
insoluble dietary fiber	29.04 ± 3.56

[a] Data are means ± SEM, $n \geq 6$. [b] $N \times 6.25$.

RESULTS AND DISCUSSION

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. sabadriffa* 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 relative molecular mass have been identified (*3*).

The brilliant red color of the flower is due to the abundant pigments, of which phenolic compounds are the main components. Following the solubility criterion, polyphenols may be classified into extractable polyphenols (EPP) and nonextractable polyphenols (NEPP) (*9*). EPPs are low and intermediate 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 scare. 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 bioaccesibility 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 2. Total Polyphenol Content in Roselle Flowers (*Hibiscus sabdariffa* L.) Dry Matter[a]

	flower
extractable polyphenols (g GAE/100 g)	2.17 ± 0.04
hydroxybenzoic acids (%)	32.6
hydroxycinnamic acids (%)	30.6
anthocyanidins (%)	30.8
flavonols (%)	5.87
nonextractable polyphenols (g GAE/100 g)	
proanthocyanidins	3.38 ± 0.06
hydrolyzable polyphenols	0.58 ± 0.03
antioxidant capacity(μmol trolox equivalents/g)	
FRAP	66.3 ± 11.2
ABTS	90.8 ± 34.8
ORAC	303.5 ± 8.2

[a] Data are means ± SEM, $n \geq 9$.

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 decoction 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 drinks. 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 3. Soluble Dietary Fiber, Polyphenols Content, and Antioxidant Capacity in Roselle Flower Beverage (*Hibiscus sabdariffa* L.)[a]

		extraction yield (%)
soluble dietary fiber (mg/100 mL beverage)	66.3 ± 3.6	28
polyphenols (mg GAE/100 mL/beverage)	66.1 ± 0.01	66
hydroxybenzoic acids (%)	28	
hydroxycinnamic acids (%)	21	
anthocyanidins (%)	29	
flavonols (%)	22	
antioxidant capacity (μmol trolox equivalents/ 100 mL beverage)		
FRAP	312 ± 7.8	89
ABTS	335.1 ± 4.6	79
ORAC	621.8 ± 1.7	42

[a] Data are means ± SEM, $n \geq 9$.

SDF consumption may influence the rheological properties of the gastrointestinal contents, the flow of digesta, the digestion, and the absorption process to varying degrees. Whereas in the large intestine SDF may serve as a substrate for fermentative microflora, the short chain fatty acids (acetic, propionic, butiric) that are produced stimulate colonic blood flow and fluid and electrolyte uptake (*31*) and produce health effects. Moreover, this SDF contains polyphenols associated with structural non-starch polysaccharides that may confer beneficial properties in the intestinal ecosystem. Only 46 mg/100 mL of polyphenols present in the Roselle beverage might be available for absorption in the small intestine. About 30% (19.8 mg/100 mL) of the polyphenols contained in the Roselle beverage were found to be associated with soluble dietary fiber. There is also some evidence that at least part of the antimutagenic effects of DF may be related to the associated phenolic compounds that reach the colon (*32*).

Regarding polyphenol content in the Roselle beverage, a high proportion (66%) of the EPPs was extracted and passed into the beverage. A similar amount of phenolic compounds in Roselle water extracts measured by Folin–Ciocaltaeu was found by Tsai et al. (*11*). Among the phenolic compounds isolated in *Hibiscus* have been quercetin, luteolin glucoside, and chlorogenic acid in addition to other previously recorded compounds like the anthocyanins delphidin-3-sambubioside and cyanidin-3-sambubioside (*3*).

The EPP concentration in the beverage was similar to that in tea, orange juice, and beer (*24*) and lower than in herbs employed in other infusions such as green tea (*Camelia sinensis*) or mate (*Ilex paraguariensis*), with 14.8 and 9.5 w/v of polyphenols, respectively (*33*). The HPLC analysis of Roselle beverage showed a higher proportion of flavones than the organic extract from the original sample, while proportion of anthocyanidins remained unchanged (**Tables 2** and **3**). The beverages are the major contributors to antioxidant capacity intake in the Spanish diet (*34*), suggesting that beverages are an important factor in the beneficial effects of dietary antioxidants. A serving of 250 mL of Roselle beverage in the Mexican diet may contribute around 165 mg of phenolic compounds. Anthocyanins and other phenolic compounds are the major source of antioxidant capacity in Roselle extract (*11*). They can scavenge free radicals, participate in regeneration of other antioxidants, and protect cell constituents against oxidative damage. For a series of values reported by Pulido et al. (*24*) and beverages data, the total extractable polyphenol determined by Folin reagent correlates with the antioxidant activity measured by FRAP ($R^2 = 0.9524$)and ABTS ($R^2 = 0.7908$). The antioxidant capacity of the Roselle beverage was measured by 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.

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