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Research Article

Coccoloba uvifera (L.) (*Polygonaceae*) Fruit: Phytochemical Screening and Potential Antioxidant Activity

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The flora of Latin America attracts gaining interest as it provides a plethora of still unexplored or underutilized fruits that can contribute to human well-being due to their nutritional value and their content of bioactive compounds. Antioxidant compounds are now of considerable interest due to their effect of preventing or delaying aging and their apparent involvement in prevention of numerous human diseases, including cancer, atherosclerosis, Alzheimer's disease, inflammation, and rheumatoid arthritis. In this work, the fruit of *Coccoloba uvifera* (L.) was investigated for their *in vitro* antioxidant capacity using two assays based on reactions with a relatively stable single reagent radical (Trolox equivalent antioxidant capacity, TEAC; and DPPH free radical scavenging assay, DPPH), two assays based on chelating of metallic cations, and one based on the reduction of oxidized species. The TEAC value on ABTS radical, DPPH scavenging activity, ion chelation, and reducing power were found to be 897.6 μM of Trolox/100 g of sample, 22.8% of DPPH free radical scavenging, 11.3% of Cu^{2+}-chelating activity, 23.9% of Fe^{2+}-chelating activity, and a Fe^{2+}-reducing power of 0.76 mg/mL, respectively. The free radical scavenging and antioxidant characteristics of *C. uvifera* may be due to the presence of diverse phytochemicals in the fruit as anthocyanins, ascorbic acid, phenolic compounds, and flavonoids.

1. Introduction

The importance of oxidation in the body and in foodstuffs has been widely recognized. Oxidative metabolism is essential for the survival of cells. Active oxygen and related species, superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide ($NO^{\bullet}$), hydrogen peroxide (H_2O_2), lipid radical ($L^{\bullet}$), lipid peroxyl radical ($LO_2^{\bullet}$), and lipid alkoxyl radical ($LO^{\bullet}$), play a vital role in biological processes of energy production, phagocytosis, and signal transduction. There is increasing evidence to show that active oxygen species may also play a causative role in various diseases such as atherosclerosis, ischemia reperfusion injury, inflammation, carcinogenesis, cataracts, brain dysfunction, immune-system decline, cardiovascular disease, and rheumatoid arthritis [1, 2]. Endogenous antioxidant enzymes, catalase, superoxide dismutase, and glutathione peroxidase defend against oxidative damage caused by active oxygen and related radicals. In addition to the enzymatic antioxidant defences, nutritional antioxidants in the diets may have protective effects to prevent oxidative stress related diseases. Low dietary intake of fruits and vegetables doubles the risk of most types of cancer as compared to high intake and also markedly increases the risk of heart disease and cataracts. In foods, it is recognized that lipid peroxidation causes deteriorations in food quality, unacceptable taste, and shortening of shelf-life. The antioxidant supplementation is a generally accepted method of prolonging the stability and storage life of food products, in particular the ones including fat. However, the artificial compounds with antioxidant properties, like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have a limited allowance for food due to their potential carcinogenicity [3].

One practical approach to solve this problem is the use of safer antioxidants from natural sources. Antioxidants are the substances able to prevent or inhibit oxidation processes in human body as well as in food products [1]. The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. The phytochemicals are a large and diverse group of secondary metabolites that exhibit strong antioxidant activity *in vitro*. They are present in fruits and vegetables, their products, leguminous plants, grains, teas, herbs, spices, and wines [4]. Fruits and vegetables are high in components like ascorbic acid, phenols, flavonoid, and anthocyanin contents; it is estimated that humans consume between a few hundred milligrams and one gram of antioxidants every day [5]. Human studies have found too that these antioxidant components appear in blood plasma, at pharmacologically active levels, after eating certain foods but do not accumulate in the plasma [6]. Currently, increasing attention has been paid by consumers to the lesser known fruits which have unusual flavours and qualities and many of which are rich in antioxidants [7]. Detailed information about the health promoting components of lesser known fruits species could lead to a better understanding of the beneficial effects and an increased consumption of these fruits, including their utilization in functional foods and as ingredients in nutraceuticals, medicine, and pharmaceuticals [8]. *C. uvifera* is a species of flowering plant in the buckwheat family, *Polygonaceae*, which is native to coastal beaches throughout tropical America and the Caribbean, including southern Florida, the Bahamas, Barbados, and Bermuda. *C. uvifera* is a sprawling evergreen shrub or small tree that reaches a maximum height of 8 m. In late summer it bears green fruit, about 2 cm diameters, in large grape-like clusters. The fruit gradually ripens to a purplish color. Each contains a large pit that constitutes most of the volume of the fruit [9]. Research on wild fruits and other wild edible plants is also intended to promote the preservation of these species, presently under threat by human activities. In addition to their nutritional value, the preservation of these fruits also has economic advantages, as there is a significant trade with some of this wild edible and medicinal fruits. Any scientific evidence for the health benefits of such wild fruits would be a value addition to the processing plants such fruits. This work will give a first appraisal of the characterization of primary compounds as well as the *in vitro* antioxidant activity of the *C. uvifera* fruit.

2. Materials and Methods

2.1. Chemicals, Raw Material, and Sampling. All reagents were of analytical grade. Ripe fruits of *C. uvifera* were collected in the coast of Yucatán, México. Fruits were botanically identified with the help of local flora [9]. The specimen (Code 2194) is deposited in the herbarium at the "Centro de Investigaciones Científicas de Yucatán, CICY," located in Yucatán, México. After reception, the fruits were selected, washed, and sanitized in chlorinated water and kept at 4°C. Pulp obtaining was carried out in an extractor. The pulp was freeze-dried at −47°C and 13×10^{-3} mbar. Until analysis, the freeze-dried pulp was stored at −20°C.

2.2. Proximal Composition. Proximate composition was determined using AOAC [10] methods: moisture content (Method 925.09); ash (Method 923.03); crude fat (Method 920.39); crude protein, using a 6.25 nitrogen-protein conversion factor (Method 954.01); and crude fiber (Method 962.09). Carbohydrate content was estimated as nitrogen-free extract (NFE).

2.3. Determination of Total Titratable Acid (TTA). Determination of TTA was performed as described in AOAC standard method 942.15 with some modifications [10]. 1 g of lyophilized pulp was dissolved in 50 mL of distilled water. The titration was accomplished with 0.1 M NaOH. After addition of several droplets of NaOH the pH value was recorded by using a pH meter. This procedure was repeated twice. The stoichiometric point was calculated and the results were expressed as citric acid equivalent.

2.4. Quantification of Phytochemical Components

2.4.1. Determination of Anthocyanins. Anthocyanins content was determined according to Salinas et al. [11]. One milliliter from the methanol extract was placed in a plastic cell for spectrophotometer and 2 mL of ethanol 96%-HCl 1.0 N was added. A calibration curve was prepared using chlorinated pelargonidin (10–50 mg/mL), using extraction solution as blank. Absorbance was measured at a 520 nm. The equation of the curve was used to calculate the concentration of anthocyanins in samples. The analysis was performed on three replicates. The total amount of anthocyanins was determined in micrograms of pelargonidin equivalents (PE)/100 g of fruit weight.

2.4.2. Determination of Ascorbic Acid. Ascorbic acid content was determined according to the official AOAC method 967.21 [10]. Ten grams of fruit was mixed with 70 mL of cold extracting solution. This cold extracting solution consisted of 30 mg of metaphosphoric acid, 80 mL of acetic acid, and distilled water added to 1000 mL and stored at 4°C. The mixture was homogenized for 20 min at room temperature and then the solution was centrifuged at 9,000 g (at 4°C) for 20 min. Several precautions were taken in order to perform all the operations under reduced light and at 4°C. To quantify ascorbic acid content, ten milliliters of ascorbic acid extracts was titrated with 2,6-dichloroindophenol solution (25 mg DCIP and 21 mg $NaHCO_3$ in 100 mL water) until light but distinct rose pink color appeared and persisted for more than 5 seconds. The 2,6-dichloroindophenol solution was standardized with ascorbic acid solution. All determinations were repeated three times and the results were expressed as mg ascorbic acid/100 g of fruit weight.

2.4.3. Determination of Total Phenolic. The phenolic compounds were determined using the Folin-Ciocalteu method,

based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolics to a blue reaction product according to the method reported by Chaovanalikit and Wrolstad [12]. One g of sample was suspended in 40 mL of 80% methanol (v/v) in a beaker. The dispersion was stirred on a magnetic plate at room temperature for 3 h. After the extraction the dispersion was centrifuged at 2500 rpm for 20 min at 10°C. The supernatant was filtered, refrigerated at 4°C, and protected from light until analysis. A volume of 0.2 mL from the methanol extract was placed in a plastic cell for spectrophotometer and 0.2 mL of Folin-Ciocalteu reagent was added and homogenized. Then 2 mL of distilled water was added and the cell was kept in dark at room temperature for 1 hour. Absorbance was measured at 765 nm. The data were calculated by comparison between a standard curve (0–500 μg/mL of gallic acid) and the absorbance of each sample. The analysis was performed on three replicates. The total amount of phenolic compounds was determined in milligrams of gallic acid equivalents (GAE)/100 g of fruit weight.

2.4.4. Determination of Total Flavonoids. The flavonoids content was determined using the aluminum chloride method reported by Dewanto et al. [13]. Briefly, 0.5 mL from the ethanol extract was placed in a plastic cell for spectrophotometer; then 1.5 mL of ethanol 95%, 0.1 mL $AlCl_3$ 10%, 0.1 mL of potassium acetate 1.0 M, and 2.8 mL of distilled water were added and the cell was kept in dark at room temperature for 30 min. Absorbance was measured at 415 nm. The data were calculated by comparison between a standard curve (0–100 μg/mL of quercetin) and the absorbance of each sample. The analysis was performed on three replicates. The total amount of flavonoid compounds was determined in milligrams of quercetin equivalents (QE)/100 g of fruit weigh.

2.5. In Vitro Antioxidant Properties

2.5.1. Trolox Equivalent Antioxidant Capacity. The $ABTS^{\bullet+}$ radical cation was produced by reacting 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) with potassium persulfate [14]. To prepare the stock solution, ABTS was dissolved at a 2 mM concentration in 50 mL phosphate-buffered saline (PBS) prepared from 4.0908 g NaCl, 0.1347 g KH_2PO_4, 0.7098 g Na_2HPO_4, and 0.0749 g KCl dissolved in 500 mL ultrapure water. If pH was lower than 7.4, it was adjusted with NaOH. A 70 mM $K_2S_4O_8$ solution in ultrapure water was prepared. The ABTS radical cation was produced by reacting 10 mL ABTS stock solution with 40 μL $K_2S_2O_8$ solution and allowing the mixture to stand in darkness at room temperature for 16-17 h before use. The radical was stable in this form for more than 2 days when stored in darkness at room temperature. Antioxidant compound content in the sample was analyzed by diluting the $ABTS^{\bullet+}$ solution with PBS to an absorbance of 0.800 + 0.030 AU at 734 nm. After adding 990 mL diluted $ABTS^{\bullet+}$ solution (A 734 nm (1/4) 0.800 + 0.030) to 10 mL of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard in PBS, absorbance was read at room temperature exactly 6 min after initial mixing. All determinations were carried out in triplicate. The percentage decrease in absorbance at 734 nm was calculated and plotted as a function of the antioxidant concentration of Trolox for the standard reference data. To calculate the Trolox equivalent antioxidant coefficient (TEAC), the slope of the absorbance inhibition percentage versus antioxidant concentration plot was divided by the slope of the Trolox plot.

2.5.2. DPPH Free Radical Scavenging Assay. The scavenging effect on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical was measured as described by Shimada et al. [15]. With some modifications, a sample solution (1.5 mL) with 20 mg was added to 1.5 mL of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at room temperature. Absorbance was measured at 517 nm. Measurement was performed at least in triplicate. The percentage of the DPPH free radical was calculated using the following equation: DPPH scavenging effect (%) = ((A0 − A1)/A0) × 100.

A0 was the absorbance of the control, where A1 was the absorbance in the presence of the *C. uvifera* pulp. The IC_{50} (concentration providing 50% inhibition) values were calculated using the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect. Samples were tested in a range of concentrations from 2.0 to 10.0 mg/mL.

2.5.3. Chelation of Metal Ions Cu^{2+} and Fe^{2+}. Cu^{2+}-chelating activity was determined using the pyrocatechol violet reagent according to Saiga et al. [16]. Briefly, 1.0 mL of sodium acetate buffer (100 mM, pH 4.9), 100 mL of Cu (II) standard solution (1.0 mg/mL), and 100 mL of sample (containing 200 μg) were mixed in a test tube. The mixture was allowed to react for 5 min at room temperature and 25 mL of a pyrocatechol violet solution (4.0 mM) was then added. Absorbance was determined at 632 nm. Copper chelating activity was calculated as follows: Chelating activity (%) = (1 − sample A632/control A632) × 100. Fe^{2+}-chelating activity was determined by measuring the formation of the Fe^{2+}-ferrozine complex. Briefly, 1.0 mL of sodium acetate buffer (100 mM, pH 4.9), 100 mL of Fe(II) standard solution (1.0 mg/mL), and 100 mL of sample (containing 200 mg) were mixed in a test tube. The mixture was allowed to react for 5 min at room temperature and 50 mL of a ferrozine solution (40 mM) was then added. Absorbance was determined at 562 nm. Iron chelating activity was calculated as follows: Chelating activity (%) = (1 − sample A562/control A562) × 100.

2.5.4. Ferric Reducing Power Assay. This method is based on the reduction of potassium ferricyanide (Fe^{3+}) in the presence of an antioxidant to (Fe^{2+}) forming the blue complex $K[Fe^{II}(CN_6)]$, which is absorbed at 700 nm [17]. First, 200 mL of sample (containing 1 mg), 500 mL of phosphate buffer (0.2 M, pH 6.6), and 500 mL of potassium ferricyanide (1%) were mixed in a test tube. The test tube was then incubated at 50°C for 20 min. Subsequently, 500 mL of trichloroacetic acid 10% (w/v) was added, and the tube was centrifuged at 3000 ×g

for 10 min. An aliquot of 500 mL of the supernatant was dissolved in an equal amount of distilled water; immediately 500 mL of ferric chloride (0.1%) was added. Absorbance was determined at 700 nm. Samples were tested in a range of concentrations from 0.2 to 1.0 mg/mL.

2.6. Statistical Analysis. All results were analyzed using descriptive statistics with a central tendency and dispersion measures. All analyses were done according to Montgomery [18] and processed with the Statgraphics Plus version 5.1 software.

Table 1: Centesimal specification of nutrients (dry weight) and titratable acidity of *C. uvifera* fruit.

Parameter	(g/100 of sample)
Protein	3.96 ± 0.48
Fat	1.82 ± 0.27
Crude fibre	3.34 ± 0.46
Ash	4.29 ± 0.36
Carbohydrates	86.59 ± 0.59
Titratable acidity*	5.9 ± 0.11
Moisture	(85.6 ± 0.16)

* Expressed in citric acid equivalents.

3. Results and Discussion

3.1. Proximal Composition and Titratable Acidity (TTA). Generally fruits are not characterized by high protein content but the protein content of *C. uvifera* (Table 1) was higher than reported for other red fruits like black currants (*Ribes rubrum*) (0.6 g/100 g of sample) [19] or blackberries (*Rubus fruticosus*) (2 g/100 g of sample) [20].

C. uvifera showed a fat content of 1.82 ± 0.27 g/100 g of sample. This result is comparable to that of berries like blackberries, blueberries, or raspberries which generally contain 1% or less of fat [19]. *C. uvifera* fruits are found to be a good source of crude fibre. The determined content was 3.34 g ± 0.46 g/100 g of sample that is about twice the amount contained in guava (*Psidium guajava* L.) (2.4 g/100 g of sample) [21]. The fibre includes plant substances that resist the action of human digestive enzymes and is divided into two fractions: water-soluble (pectin, gum) and water-insoluble (cellulose, lignin, and some of the hemicellulose). The latter is mainly credited for regulating bowl movement whereas the soluble fraction is chiefly involved in lowering blood cholesterol and glucose adsorption [21]. *C. uvifera* fruits are found to be a good source of carbohydrates (86.59 ± 0.59 g/100 g of sample). Therefore, *C. uvifera* fruit pulp may be a valuable source of nutrients.

TTA of *C. uvifera* (Table 1) is comparable to that of ripe blackberries (5.78 equivalents of citric acid/100 g of fruit) [20]. The TTA is directly related to the organic acid content of the fruit. Organic acids are of great significance in plants as intermediates in the metabolic processes of the fruit; these acids are directly involved in growth, maturation, and senescence [22]. High values of TTA indicate that fruit is not ripe; on the contrary overripe fruits have very low levels of TTA. The state of maturity also influences the content of vitamins and phytochemicals related with the antioxidant properties of the fruit [23]. The TTA value obtained for the *C. uvifera* fruit pulp indicates that the fruit was processed in an adequate state of maturity, whereby the content of vitamins and phytochemicals may be the most appropriate to generate antioxidant activity.

3.2. Quantification of Phytochemical Components

3.2.1. Determination of Anthocyanins. The representative phenolic colorant anthocyanin is a subgroup of flavonoids that contain $C_3C_6C_3$ carbon skeleton and cover a broad range of colors including blue, purple, violet, magenta, red, and orange. Even though flavonoids release colors as well, anthocyanins are the most broadly distributed pigment in the plant world [24]. Anthocyanins differ in the number of hydroxyl and/or methoxy groups present and sugars such as glucose, galactose, arabinose, and xylose are attached to the 3 positions in the C ring [25]. Anthocyanins are involved in a wide range of biological activities and may affect positively the health. Many of the biological properties are closely associated with the antioxidant activity of anthocyanin pigments [26]. Antioxidative properties of anthocyanins arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol-derived radicals to stabilize and delocalize the unpaired electron, and from their ability to chelate transition metal ions [24]. Total anthocyanin content in *C. uvifera* pulp fruit was measured using a spectrometric method. An aliquot of sample was properly diluted into a spectrometric linear range for chlorinated pelargonidin (10–50 mg/mL), using extraction solution as blank. Anthocyanin content of *C. uvifera* pulp fruit was reported as μg of pelargonidin equivalents/100 g of fruit weight (Table 2). The content of anthocyanins of *C. uvifera* fruit pulp is comparable to that of strawberry (24.0 mg/100 g) [27]. It has been estimated that daily ingest of anthocyanins is 12.5 mg and anthocyanin remains intact when passing from the digestive tract to the blood circulation of mammals [6]. This is extremely important considering that anthocyanins not only act as antioxidants but also reduce the risk of coronary heart disease, inhibit platelet aggregation, and protect arterial endothelial cells. In addition, these pigments could decrease the risk of cancer, reduce inflammatory process, and modulate immune response [28]. The obtained results indicated that the fruit of *C. uvifera* could be considered a good source of dietary anthocyanins.

3.2.2. Determination of Ascorbic Acid. Ascorbic acid (vitamin C or ascorbate) is a six-carbon lactone that is synthesized from glucose by many animals. However, humans are unable to synthesize vitamin C. When there is insufficient vitamin C in the diet, humans suffer from the potentially lethal deficiency disease scurvy. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables [29]. Ascorbic acid is an electron donor (reducing agent or antioxidant), and probably all of its biochemical and molecular functions

Table 2: Phytochemical components of *C. uvifera* fruit.

Phytochemical component	Value
Anthocyanins (mg of pelargonidin equivalents/100 g of fruit weight)	32.94 ± 3.29
Ascorbic acid (mg of ascorbic acid equivalents/100 g of fruit weight)	6.25 ± 0.87
Total phenolic compounds (mg of gallic acid equivalents/100 g of fruit weight)	177.23 ± 9.45
Total flavonoids compounds (mg of quercetin equivalents/100 g of fruit weight)	9.67 ± 1.12

can be accounted for by this function. Ascorbic acid acts on the one hand as an important water-soluble antioxidant in biological fluids by scavenging physiologically relevant reactive oxygen species. In addition to this direct antiradical capacity, ascorbic acid is able to regenerate other antioxidants like α-tocopherol, glutathione, urate, and β-carotene from their respective radical species. On the other hand, ascorbic acid exerts also regulatory functions on a cellular level by influencing gene expression and apoptosis and can act as a cofactor maintaining the activity of a number of enzymes by keeping metal ions in the reduced state [30]. The ascorbic acid content of *C. uvifera* pulp fruit was determined by titrimetric method using 2,6-dichloroindophenol and reported as ascorbic acid/100 g of fruit by reference to a standard ascorbic acid solution (Table 2). The *C. uvifera* fruit content of ascorbic acid was comparable to that of apple "green" (6.0 mg/100 g), banana (3.1 mg/100 g), coconut (3.3 mg GAE/100 g), and water melon "light green" (6.8 mg/100 g) [31]. The obtained results indicated that the content of ascorbic acid of the *C. uvifera* fruit is relatively low. However the consumption of fruits is beneficial and the health effects are ascribed in part to ascorbic acid content [32]. Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. The ascorbyl free radical can be converted back to reduce ascorbate by accepting another hydrogen atom or it can undergo further oxidation to dehydroascorbate. Dehydroascorbate is unstable but is more fat soluble than ascorbate and is taken up 10–20 times more rapidly by erythrocytes, where it will be reduced back to ascorbate by NADPH from the hexose monophosphate shunt; thus mechanisms exist to recycle vitamin C in human organism [29]. Therefore it is important to consider the contribution of ascorbic acid in the antioxidant activity of *C. uvifera* fruit.

3.2.3. Determination of Total Phenolic. Phenolic compounds are commonly found in both edible and inedible plants and have been reported to have multiple biological effects, including antioxidant activity. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as number and location of hydroxyl groups [33]. The total phenolic content of *C. uvifera* pulp fruit was determined using Folin-Ciocalteu method and reported as gallic acid equivalents by reference to standard curve (Table 2). The amount of extractable polyphenols varied greatly among the fruit species. According to Vasco et al. [34], fruits can be classified into three categories by their polyphenol content: low (<100 mg GAE/100 g), medium (100–500 mg GAE/100 g), and high (>500 mg GAE/100 g). According to the above classification the *C. uvifera* pulp fruit would be classified with a medium content of polyphenols comparable to that of apple "Gala" (132 mg GAE/100 g), orange (217 mg GAE/100 g), kiwi fruit (273 mg GAE/100 g), and pink grapefruit (425 mg GAE/100 g). Polyphenol supply for the human organism is affected, not only by their concentrations in the raw material, but also by the amount of consumption. Besides, these compounds are more active in human organism in the presence of vitamin C. It is also thought that polyphenols present in fruit have stronger effects because saccharides enhance their assimilation. Saccharides bound to polyphenols act as hydrogen ligands in hydroxyl groups of these compounds. As antioxidants, dietary polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress. Numerous studies on animal models have shown that, when added to the diet, they limit the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, and osteoporosis [35]. The obtained results indicated that the fruit of *C. uvifera* could be considered as a good source of dietary polyphenols.

3.2.4. Determination of Total Flavonoids. Flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. In plants, these compounds afford protection against ultraviolet radiation, pathogens, and herbivores [36]. Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. By virtue of their capacity to inhibit LDL oxidation, flavonoids have demonstrated unique cardioprotective effects [37]. A protective role in the diet of humans has also been indicated in some large, prospective studies. For example, high flavonoid intake predicted lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men and reduced the risk of coronary heart disease by 38% in postmenopausal women. The Zutphen Elderly Study demonstrated an inverse relationship between consumption of catechin, a predominant flavonoid in tea, and ischemic heart disease mortality in a cohort of 806 men [38]. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases [39]. The total flavonoids content of *C. uvifera* pulp fruit was determined using the aluminum chloride method and

Table 3: Antioxidant properties of *C. uvifera* fruit pulp compared with standard antioxidants.

Parameter	*C. uvifera*	Trolox [49]	BHT* [49]
TEAC (μM of Trolox/100 g of sample)	897.6 ± 22.8	IC_{50} = 2.08 μg/mL	IC_{50} = 1.66 μg/mL
DPPH (% of free radical scavenging)	22.8 ± 0.44	48	55
IC_{50} (μg/mL)	4.4 ± 0.87	5.28	1.66
Cu^{2+}-chelating activity (% of chelating activity)	11.3 ± 0.65	34	6
Fe^{2+}-chelating activity (% of chelating activity)	23.9 ± 0.51	14	11
Ferric reducing power, IC_{50} (μg/mL)	760 ± 4.0	19.04	20.62

*Butylated hydroxytoluene.

the total amount of flavonoid compounds was determined in milligrams of quercetin equivalents (QE)/100 g of fruit weight (Table 2). The *C. uvifera* fruit content of total flavonoids was comparable to that of apple (7.0 mg/100 g), black currant (6.8 mg/100 g), and elderberry (10.5 mg GAE/100 g) [40]. It is now widely accepted that dietary flavonoids may play an important role in protecting the body against chronic diseases, such as cancer, cardiovascular diseases, and diabetes mellitus [41]. The obtained results indicated *C. uvifera* fruit is a good source of dietary flavonoids.

3.3. Antioxidant Properties. Several methods have been developed for the assessment of the antioxidant efficiency. Because many active species and reaction mechanisms are involved in oxidative stress process; no simple universal method can be applied for accurate and quantitative measurement of antioxidant capacity [42]. Generally, in these methods a radical is generated and the antioxidant capability of a sample against the radical is evaluated. In the present study, the antioxidant activity of the *C. uvifera* fruit pulp was determined using ABTS and DPPH radical scavenging assays, metal chelating assay, and ferric reducing antioxidant power assay.

3.3.1. Trolox Equivalent Antioxidant Capacity. The ABTS assay is based on the inhibition by antioxidants of the absorbance of the radical cation $ABTS^{\bullet+}$. The use of Trolox as a standard allows the assay to be called Trolox equivalent antioxidant capacity (TEAC). ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants [43]. The radical cation $ABTS^{\bullet+}$ is soluble in aqueous and organic solvents, making the assay a suitable method for determining the hydrophilic and lipophilic antioxidant capacity of extracts and biological fluids [44]. The *C. uvifera* fruit pulp efficiently scavenged ABTS radicals generated by the reaction between 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and potassium persulfate (Table 3).

The ABTS radical scavenging activity of *C. uvifera* (897.6 μM of Trolox/100 g) is comparable to that of other fruits like guava (*Psidium guajava*) (820 μM of Trolox/100 g), grape (*Vitis vinifera*) (920 μM of Trolox/100), or Acai (*Euterpe oleracea*) (940 μM of Trolox/100 g) [45].

3.3.2. DPPH Free Radical Scavenging Assay. The radical DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition, brought about by various additives. A freshly prepared DPPH solution exhibits a deep purple color with a maximum absorption at 517 nm. This purple color generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them to a colorless/bleached product, DPPH-H (i.e., 2,2-diphenyl-1-hydrazine or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm [46]. It is also important to note that the DPPH test only recognizes free radical scavenging effects and not prooxidant activity. The percentage of DPPH radical scavenging of the *C. uvifera* (22.8%) is showed in Table 3. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compound reduce and decolorize DPPH by their hydrogen donating ability. It appears that *C. uvifera* possesses hydrogen donating abilities to act as an antioxidant. Surinrut et al. [47] reported high antioxidant activity (IC_{50} from 1.1–9.6 μg/mL) in fruits like mulberries (*Morus rubra*), mango (*Mangifera indica*), carambola (*Averrhoa carambola*), and guava (*Psidium guajava*). The DPPH radical scavenging IC_{50} (4.4 μg/mL) of *C. uvifera* pulp is comparable to that of other fruits.

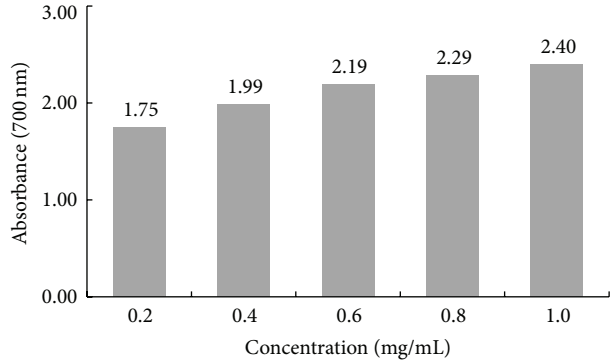

Figure 1: Ferric reducing power of *C. uvifera* fruit pulp.

3.3.3. Chelation of Metal Ions Cu^{2+} and Fe^{2+}. Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity plays a significant role in

antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. At a concentration of 0.2 mg/mL, the *C. uvifera* lyophilized juice exhibits chelating effects of 11.3% and 23.9%, for Cu^{2+} and Fe^{2+}, respectively. It was confirmed in numerous studies that, in addition to direct free radical scavenging, flavonoids exert antioxidant activity through interactions with the reduced form of transition metals, primarily Fe(II), Fe(III), and Cu(I), which participate in reactions generating free radicals [48]. The results of the present study suggest that the *C. uvifera* fruit pulp exhibits chelation activity on cuprous and ferrous ions probably due to their content of flavonoids.

3.3.4. Ferric Reducing Power Assay. The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power. Figure 1 presents the reductive capabilities of the *C. uvifera* juice. In the concentration range investigated, the lyophilized juice demonstrated reducing power that increased linearly with concentration. At 0.4, 0.8, 1.2, 1.6, and 2.0 mg/mL, reducing power of *C. uvifera* pulp were found to be 1.75, 1.99, 2.19, 2.29, and 2.40, respectively. The IC_{50} value was found to be 0.76 mg/mL (760 μ/mL). The reducing power of the *C. uvifera* pulp might be due to their hydrogen donating ability.

The beneficial effects of fruits are attributed largely to substances proven to be effective free radical scavenging and having antioxidant properties, such as anthocyanins [11], ascorbic acid, polyphenolic [12], and flavonoid [13] compounds. At this point it is good to recall the difference between an antioxidant and a radical scavenger, as often these terms are both used in antioxidant literature. In principle, any radical scavenger can be an antioxidant if it terminates radical chain propagation. Radical scavengers are the largest class of antioxidants. In addition antioxidant classes such as metal chelators, oxygen scavengers, UV light absorbers, and enzymatic antioxidants are recognized. Possibly, *C. uvifera* fruit pulp contains high amounts of phytochemicals, which could react with radicals to stabilize and terminate radical chain reactions.

4. Conclusion

Primary compounds, phytochemicals, and antioxidant properties of *C. uvifera* fruit pulp are being reported here for the first time. This fruit is a good source of nutrients and shows *in vitro* antioxidant properties comparable to those of conventional fruits. The antioxidant effects appeared to be due to the contents phytochemicals like polyphenols, flavonoids, anthocyanins, and ascorbic acid. Total phenolic compounds in *C. uvifera* fruit pulp seemed to be the major contributors. Mexico is one of the countries that have a rich diversity of underutilized fruits that grow wild in the region of Yucatan Peninsula. Some of the underutilized fruits are rarely eaten, unknown, and unfamiliar. However, underutilized fruits have not received much attention as antioxidant sources compared to commercial fruits like guava, papaya, and pineapple. This could be due to their lack of popularity among local communities, lack of information on nutritional compositions and physical qualities, and the lack of promotional campaign for these fruits. This research improves the knowledge of plants from Mexico and will promote their appreciation, although more studies must be carried out to identify and quantify the phytochemicals associated with their antioxidant activity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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