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Original Article

Physicochemical and *in Vitro* Antioxidant Properties of Juice and Cake Filters from *Carissa edulis Vahl* Fruits

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Abstract

Background and objectives: *Carissa edulis* (Apocynaceae) is a wild fruit species highly consumed by Cameroonian populations because of its many biological effects. However, few studies so far have addressed the nutritional, antioxidant, and physicochemical properties of this plant.

Methods: Juice and cake powders obtained from *C. edulis* fruits were examined for their contents in macronutrients, micronutrients, and phenolic compounds through conventional methods. Then, the *in vitro* antioxidant properties were assessed using the 1,1-diphenyl-2-picrylhydrazyl radical scavenging, total reducing power, and the total antioxidant capacity assays.

Results: The results showed that cakes displayed significantly higher fat (22.68 ± 2.16 vs 5.06 ± 0.43 g/100 g dry weight (DW)), carbohydrates (39.25 ± 1.16 vs 19.29 ± 0.55 g/100 g DW), protein (1.32 ± 0.56 vs 0.23 ± 0.13 g/100 g DW), zinc, copper, and calcium levels compared to juice. However, their ash (0.28 ± 0.02 vs 0.31 ± 0.02 g/100 g DW), moisture (5.67 ± 0.53 vs 14.40 ± 1.36 g/100 g), carotenoids, and Vitamin C levels were significantly lower. The phenolic content in the juice was generally lower ($p < 0.05$) than in the cake. Polyphenols, flavonoids tannins, and anthocyanins were respectively the most quantitatively important compounds. On the other hand, the study of the antioxidant activity revealed that the cake had higher antioxidant activities.

Conclusions: Taken all together, the results showed that the cake of the *C. edulis* fruits has higher nutritional value, bioactive compound levels, and antioxidant potentials than juice which merits further consideration as food supplements.

Keywords: *Carissa edulis*; Juice; Cake; Antioxidant activity; Chemical composition.
Abbreviations: AAE, ascorbic acid equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DW, dry weight; FW, fresh weight; GAE, gallic acid equivalent; LABBAN, laboratoire de biophysique, biochimie alimentaire et nutrition; PVPP, polyvinylpolypirrolidone; RE, rutin equivalent; TAC, total antioxidant capacity; TRP, total reducing power.
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Introduction

The edible wild fruit species are those that are neither produced nor domesticated but are available in their natural environment and used as food and socioeconomic wellbeing sources, particularly by rural impoverished communities.[1,2] Botanical investigations and literature emphasize the abundance, diversity, and economic worth of wild edible fruit species throughout the world. In Cameroon, edible wild fruits are valued and regularly sold in markets[3] throughout the year. The indigenous populations realize and use their nutritional, medicinal, therapeutic, and indus-

trial possibilities. They also feed on small local businesses and generate the income needed for survival in many households.[3] Edible fruits, in general, are important for a balanced diet, because they serve as food supplements and provide humans with very essential nutrients such as dietary fibers, proteins, sugars, and vitamins (particularly Vitamin C in some fruits), as well as health-promoting phytochemicals and major minerals constituents.[4]

Carissa edulis Vahl (Apocynaceae) is a multi-stemmed, much-branched prickly evergreen shrub or small tree, that may grow up to 6 m tall and produce a dense canopy. Because of its tiny size, seedless/small seed, and ability to be eaten whole, this plant is sometimes referred to as a berry. Its fruits are fleshy, oval, 6–11 mm in diameter, with red to purple blackberries and two- to four-seeded.[5] *C. edulis* fruits are vibrantly colored, becoming red, purple, and purplish-black when ripe. Furthermore, due to their nutritional content, therapeutic value, and application in many processing commodities, such as drinks, jellies, and syrup,[6,7] these wild fruits are usually harvested and consumed uncooked fresh in most rural areas. Indeed, these wild fruits consumed by locals are a good source of nutrients, and given their low cost and widespread availability, they should be promoted for commercial exploitation. In Sudan and Kenya, the fruits of *C. edulis* are used to make vinegar through fermentation and jam. The intense red-purple and purple coloration of these berries indicate the richness of their fruits in anthocyanins which are natural antioxidants.[8] Aside from flavonoids, fruits, and vegetable coloring, it can also be caused by the presence of other chemicals such as carotenoids and chlorophylls.[9] Several investigations conducted on *C. edulis* fruits have shown a variety of bioactive phytochemicals including polyphenolic antioxidants, vitamins, minerals, and a variety of nutraceutical, and biological effects including antioxidant properties.[10–12] Thus, consuming nutritious components is a critical approach for regulating and avoiding various diseases as well as boosting the usage of natural substances. Nonetheless, to the best of our knowledge, no research has been undertaken on the nutritional value, phenolic and antioxidant characteristics of *C. edulis* fruit processed goods. Although these fruits are regularly processed into juice and cake, an investigation of the physicochemical, antioxidant properties, as well as phenolic contents of the fruit juice and cake, will undoubtedly provide insight into their nutritional and medicinal potential. The current study solely looked at the nutritional value, bioactive components, and antioxidant properties of juice and cake made from fruits of *C. edulis* fruits taken in the dark red stage.

Material and methods

Plant material collection

During the rainy season, from July to August 2019, wild edible fruits of *C. edulis* were obtained at the correct edible stage in Rhumzou village in Cameroon's Far North region. These fruits were chosen because they are most appreciated and consumed, and they are also commonly processed into by-products such as juice, for sale in local markets. At the peak of ripeness, the dark red stage, fresh fruits were gathered. They were preserved in cardboard packaging and sent to the "Laboratoire de Biophysique, Biochimie Alimentaire et Nutrition (LABBAN)", at the University of Ngaoundere, Cameroon's National School of Agro-Industrial Sciences.

Methods

Processing fruits into dehydrated juices and cakes

The wild edible fruits of *C. edulis* were hand-cleaned from foreign bodies (inorganic materials, dirt, and dust particles) in the laboratory before being rinsed with tap water. The fruit juices were extracted using a hand-operated pulp extractor (Singsung; model: BL500; voltage: 240 V; frequency: 60 Hz; wattage: 500 W) that allows shredding with a stainless-steel shredder and removing the edible portion of the fruits (free and bound fractions around the fruit seeds) and then finely crushing it. The fruit's seeds were removed, and the resulting pastes (homogenate) were collected and wrapped in muslin before being physically pressed to extract fruit juice. The collected filtrate which is fruit juice and the cake filter were set aside. As a result, the crude/pulpy liquids and cake produced were maintained in a freezer at −18°C for 24 h before being freeze-dried at −60°C for 48h under the pressure of 10 Pa. The juice and cake powders were stored in polyethylene bags at 4°C until they were analyzed. After crushing the pulp, the paw was manually squeezed in the muslin to drain the juice and get the cake. The resulting juice was then filtered using Whatman Grade 4 filter paper. The cakes and juices obtained were freeze-dried (−55°C, 1 mbar) for improved preservation for future studies. The freeze-dried powders were packed in polyethylene bags and kept at 4°C until analysis. Figure 1 depicts the production technique for the cake and juice powders. Recoverable juice from plant fruits was represented as a percentage of fresh weight (mL/100 g FW), and the cake filter was expressed as g per 100 g FW. In the case of acquired juice and cake powders, Equation 1 yields the dry product yield (juice and cake powders). The yield of juice and cake powder was determined using the following equation:

$$\text{Yield } (\%) = \frac{\text{mass of lyophilizate}}{\text{initial fruits mass}} \times 100 \quad (1)$$

Determination of macronutrients contents

The biochemical content of juice and cake powders was assessed using conventional procedures. Moisture content was determined by weighing 5 g of a powder sample after drying it in an oven at 103 ± 2°C for 24 h until it reached a constant weight.[13] In addition, the ash content was determined as previously described[13] by incinerating 5 g of powder in a muffle furnace at 550°C for around 12 h or until a steady weight of greyish white ash was achieved. After mineralization in concentrated sulfuric acid and colorimetric measurement,[14] the total protein content was measured. The crude fat content of a 5 g powder sample was determined using the Soxhlet extraction technique for 8 h with hexane as the extraction solvent as previously reported.[15] Carbohydrate content was assessed using sulfuric acid-based techniques.[16]

Determination of some minerals

The mineral composition of ash samples generated by incineration of powder samples in a muffle furnace at 550°C[17] has been determined. One gram of ash was dissolved in 10 mL of 1.5 N hydrochloric acid and the mixture was heated on a hot plate until completely dry. The solution was then built up to 25 mL in a calibrated flask with a few drops of H_2O_2 and 5 mL of de-ionized

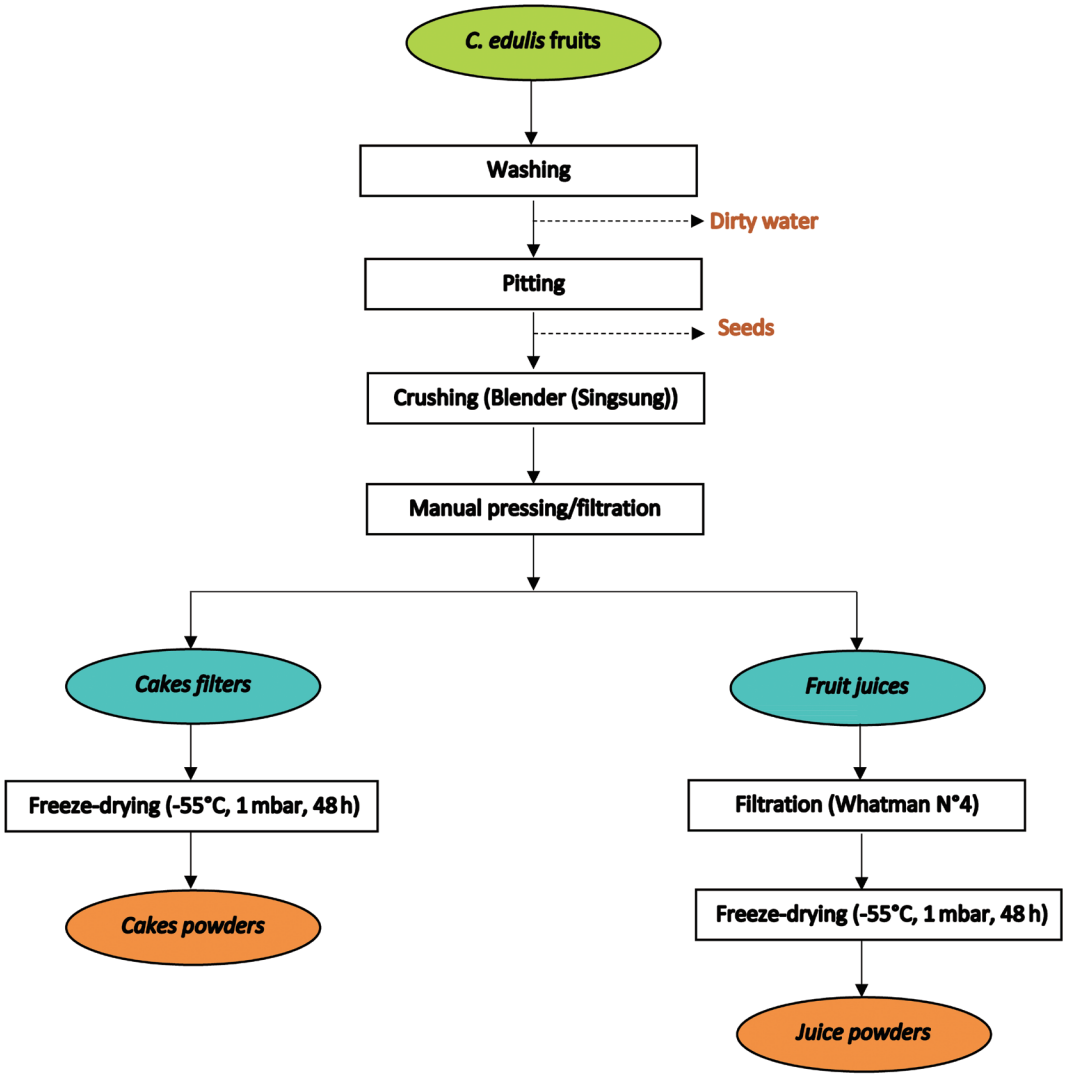

Fig. 1. Production of juice and cake powders.

water. The resultant solutions were used to determine the concentrations of iron, copper, calcium, and zinc using atomic absorption spectrometry.

Determination of total carotenoids and vitamins

Total carotenoids

Total carotenoids were extracted with a hexane-acetone mixture: 30/70 (v/v), and then the optical density of the resulting solution was read between 430 and 450 nm to determine the maximal absorbance using a spectrophotometer.[18] The maximum optical density was used to calculate the concentration of total carotenoids in the sample. The total carotenoid content was estimated using the equation below:

$$C = \frac{F \times DOmax}{196 \times M} \quad (2)$$

where DOmax is the optical density for maximum absorption; F is the dilution factor; M is the sampled mass; and C is the concentration of total carotenoids. The total carotenoids amount (m_{car}) in the diluted solution for the spectrophotometer was calculated based on the following formula:

$$m_{car} = C \cdot V \quad (3)$$

For 100 g dry matter with a water content TE, the total carotenoid content TC is given by the relation:

$$TC = \left[\frac{100 \times m_{car}}{100 - TE}\right] \times 100 \quad (4)$$

Vitamin C

The Vitamin C concentration was measured using a titrimetric assay with 2.6-Dichlorophenolindophenol as an indicator as reported previously.[19] For the extraction, 10 g of each powder were precisely weighed and added to 20 ml of 95% acetic acid. The stand-

ard solution of Vitamin C (0.01mg/mL) was placed in a burette and progressively added drop by drop into a beaker containing 1 mL of 2.6-Dichlorophenolindophenol until the solution turned pink. The reaction was continued until the content of the beaker gets completely discolored. Then, the volume of Vitamin C that allowed the discoloration of the beaker solution was noted, and the Vitamin C content of the sample was estimated using the following equation:

$$\text{Vit C} = \frac{XV}{PV'} \quad (5)$$

where X is the volume of Vitamin C that allowed the discoloration of the beaker solution; V is the total volume of extract; V′ is the volume of extract that allowed the discoloration of the beaker solution; P is the sample weight. In relation with dry matter, Vitamin C content was calculated as follows:

$$\text{Vit C} = \frac{XV/PV'}{100 - H^0} \times 100 \left(\text{mg} / 100 \text{ g DM}\right) \quad (6)$$

where H is the water content, and DM is the dry weight.

Determination of phenolic bioactive compounds

Extraction of phenolic compounds

The polyphenols were extracted using the procedure earlier described by Deli *et al*. (2019)[20] wherein dried samples are macerated with solvent to reduce sample degradation. Briefly, 2 g of the pulverized freeze-dried juice and cakes were solubilized in 20 mL of methanol/water solvent system (70/30, v/v). The mixture was stirred at 300 rpm at room temperature for 24 h before being filtered with a Whatman Grade 1 filter paper to remove insoluble residues. Finally, the recovered supernatant was diluted to 15 mL with the extraction solvent and kept at 4°C for further analysis.

Determination of total phenolic content

The technique of Li *et al*. (2007) was used to estimate total phenolic content (TPC) with minor changes.[21] In short, 20 µL of hydro methanolic extract of plant powders was combined with 2,980 µL distilled water. Then, 500 µL of 10% (v/v) Folin-Ciocalteu reagent and 400 µL of a saturated solution of sodium carbonate Na_2CO_3 (20%, w/v) were added. The mixture was well agitated and incubated at room temperature for 30 min in the dark to equilibrate. A spectrophotometer was used to measure the absorbance of the solution at 760 nm. Using standard gallic acid solutions (40, 80, 120, 160, 200, 240, and 280 g/L), a calibration curve (R^2 = 0.98) was created. The TPC was therefore represented in milligram gallic acid equivalents per gram dry weight (mg GAE/g DW) of the sample.

Determination of total tannins content

Tannin content was calculated by subtracting TPC from non-tannin phenolic content in the sample. One mL of the diluted juice sample was combined with 1 mL of distilled water and 100 mg polyvinylpolypirrolidone (PVPP) to measure the non-phenolic content. The mixture was vortexed and then centrifuged for 10 min at 3,000 rpm after being left for 15 min at 4°C. Similarly, the non-tannin phenolic content was determined in the supernatant.

$$\text{Tannins (mg GAE/g DW)} = \text{total phenolic content} - \text{non-tannin compounds} \quad (7)$$

Determination of total flavonoid content

The total flavonoid content was assessed using the technique described by Dewanto *et al*. (2002).[22] In brief, 0.1 mL of filtered hydroethanolic extract was added to 2.4 mL of distilled water followed by 0.15 mL of 5% (w/v) sodium nitrite (Na_2NO_2) solution. After 6 min, 0.3 mL of 10% aluminium chloride ($AlCl_3 \cdot 6H_2O$) (w/v) was added. After another 5 min at room temperature, 1 mL 4% (w/v) sodium hydroxide (NaOH, 1 M) was added. The absorbance of the solution was measured at 510 nm using UV/visible spectrophotometry against the extraction solvent as a blank. A calibration curve (R^2 = 0.99) was plotted from different concentrations of rutin as standards (20, 40, 80, 100, 120, and 140 g/L), and the findings were reported in milligrams rutin equivalents per gram of dry weight (mg RE/g DW).

Determination of total anthocyanin content

Anthocyanin extraction was carried out using a previously reported technique.[23] One gram of powder sample was added to 10 mL of solvent (0.1 Methanol/HCl, 85/15, (v/v)). The mixture was then centrifuged at 6,000 g for 10 min at 4°C. The supernatant was collected and the total anthocyanin content was determined using the pH-differential technique. In this regard, an aliquot (100 µL) of adequately diluted samples was placed in the tubes containing 4,900 µL of 0.025 M potassium chloride buffer at pH 1.0 and 4,900 µL of 0.4 M sodium acetate buffer at pH 4.5 respectively. The mixtures were stirred and incubated for 15 min at room temperature (25°C). Absorbance was measured both at 520 and 700 nm using a UV-Vis spectrophotometer against a blank containing distilled water. The results are given in milligram per 100 grams of powder, according to the following equation:

$$\text{CA} = \frac{A \times MW \times DF \times V}{\varepsilon \times L \times w} \times 100 \quad (8)$$

where CA is the concentration of anthocyanins (mg/100 g of dry powder); A is the absorbance difference (A = [A520 nm − A700 nm] pH=1 − [A520 nm − A700 nm] pH = 4.5); MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mole); DF is the dilution factor; V is the total volume of extract (mL); ε is the coefficient of molar extinction for cyanidin-3-glucoside (26,900 L/mole-cm); L is the cell width (1 cm); w is the weight of the sample used in the extraction (g), and 100 is the conversion factor for obtaining mg/100 g of sample.

In vitro antioxidant activities

DPPH radical scavenging activity

The antioxidant activity was initially assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method,[24] in which the extract's electron-donating capacity was quantified by whitening of the purple-colored solution of DPPH cation radical. Briefly, 2 mL of 0.1 mM DPPH methanolic solution were added to 0.5 mL hydro methanolic extract of plant sample at various concentrations (0.025, 0.05, 0.1, 0.5, 1, 5, 10, 100 mg/mL). The mixture was properly mixed before

Table 1. The juice and cakes content and dry matter of *C. edulis* fruits

Contents/Powder	Values
Cakes contents (g/100 g fruits)	24.45 ± 1.12
Juice contents (g/100 g fruits)	60.64 ± 1.59
Juice powder (g/100 mL of juice)	09.28 ± 0.59
Cake powder (g/100 g fresh cake weight)	64.14 ± 2.43

Means ± SD (n = 3).

being incubated in the dark for 1 h at room temperature. The absorbance of the mixture was then measured at 517nm using UV/visible spectrophotometry. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity was estimated based on the proportion of scavenged DPPH radicals using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (9)$$

Finally, the rate of free radical scavenged versus log concentrations of antioxidant (or plant extracts) was plotted to create a curve from which the value of IC50 (µg/mL), which is inversely proportioned to the antioxidant activity was calculated. Ascorbic acid was utilized as a reference standard at the same concentrations as plant extracts

Total reducing power

The total reduction power of the plant extracts was determined using the method published previously by Oyaizu (1986).[25] In this approach, antioxidant chemicals in the extracts generated a colorful complex with potassium ferricyanide, trichloroacetic acid, and ferric chloride, which was detected and quantified at 700 nm. The rise in absorbance of the reaction mixture indicates the reducing power of the samples. Extracts of Vitamin C at various concentrations (0.02; 0.04; 0.06; 0.08; 0.1 mg/mL) were combined with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferrocyanide and incubated at 50°C for 20 min. Then, the mixture was treated with 2.5 mL of 10% trichloroacetic acid and centrifuged at 800 rpm for 10 min. Following that, 2.5 mL of supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$). Finally, the absorbance of each mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power. The Ferric reducing power was calculated using the calibration line obtained by concentrations of the Vitamin C solution that varied. The results of the ferric reducing power were represented in mg equivalent ascorbic acid (mg EAA/100 DW).

Total antioxidant capacity

The total antioxidant capacity of the tested extracts was evaluated using the phosphomolybdate method with ascorbic acid as a standard.[26] An aliquot of 0.5 mL of extract (100 µg/mL) solution was combined to 5 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were sealed and incubated in a water bath at 95°C for 90 min. They were then cooled to room temperature and the absorbance of each solution was measured at 695 nm against a blank. Under the same circumstances, a typical blank comprising 5 mL of the reagent solution and an adequate volume of the solvent was incubated. The total antioxidant capacity of the extracts was expressed in mg equivalent per g of powder (mg AAE/g DW).

Table 2. Macronutrients, ash, and moisture contents *C. edulis* juices and cakes

	Values	
	Juice	Cake
Moisture, %/100 g DW	14.40 ± 1.36	5.67 ± 0.53***
Ash, %/100 g DW	0.31 ± 0.02	0.28 ± 0.02*
Fat, %/100 g DW	5.06 ± 0.43	22.68 ± 2.16***
Carbohydrates, g/100 g DW	19.29 ± 0.55	39.25 ± 1.16***
Total proteins, g/100 g DW	0.23 ± 0.13	1.32 ± 0.56*

Values were expressed as means ± SD (n = 3). Values from the cake group were statistically compared against the juice group. (*$p < 0.05$; ***$p < 0.001$) using the Duncan's multiple comparison test.

Statistical analysis

The obtained data were collected in an Excel file and represented as means ± standard deviations (SD). Analysis of variance (ANOVA) was used to assess if there were statistically significant changes in nutritional composition, phytochemical content, and antioxidant activity ($p < 0.05$). The level of significant difference in the two means was determined using Duncan's multiple comparison tests. Finally, Stat graphics Centurion 16.1 was used. Also, Sigma plot 11.0 was used to plot the graphs.

Results

Extraction yield of juice and cakes from the C. edulis fruits

Table 1 shows the juice and cake extraction yields from the fruits of *C. edulis*. The values obtained in this table show that *C. edulis* is very juicy with juice percentages of 60.64 ± 1.59% compared to 24.45 ± 1.12% in the cakes.

Macronutrient, ash, and moisture contents of juice and cake powders from C. edulis fruits

Table 2 shows the macronutrient composition analysis of juice and cake powder from *C. edulis* fruits. As indicated in the table, the cake had a significantly ($p < 0.05$) greater macronutrient content than the juice although the juice had higher ash and moisture content. However, carbohydrates, lipids, and total proteins were the most prevalent macronutrients in both samples.

Micronutrient, Vitamin C, and carotenoid contents

Table 3 summarizes the mineral, carotenoid, and Vitamin C content. The juice had much more Vitamin C and carotenoids than the cakes, while the cakes had more iron, zinc, calcium, and copper. Calcium was the most abundant micronutrient in the cakes and juice, followed by Vitamin C. Calcium levels varied from 43.78 ± 3.32 mg/100 g DW for the juice to 80.52 ± 6.50 mg/100

Table 3. Total carotenoids and micronutrients contents of juice and cake powders from *C. edulis* fruits

	Values	
	Juice	**Cake**
Total carotenoids	0.80 ± 0.01	0.73 ± 0.28*
Vit C	32.57 ± 2.41	24.91 ± 1.56**
Iron	1.34 ± 0.07	1.47 ± 0.39
Zinc	1.88 ± 0.47	4.28 ± 0.30**
Copper	0.4 ± 0.11	1.12 ± 0.84*
Calcium	43.78 ± 3.32	80.52 ± 6.50***

Values were expressed as means ± SD (n = 3). Values from the cake group were statistically compared against the juice group. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) using the Duncan's multiple comparison test.

g DW in cakes, while Vitamin C levels ranged from 32.57 2.41 mg/100 g DW to 24.91 ± 1.56 mg/100 g DW in cakes. Higher zinc contents (4.28 mg/100 g DW) were found in the cakes ($p < 0.05$). Furthermore, the iron level of the juice was 1.34 mg/100 g DW vs. 1.47 mg/100 g DW for the cakes.

Bioactive compounds content of C. edulis juices and cakes

The total polyphenol, flavonoid, anthocyanin, total carotenoids, and tannin content of the study's fruit powders are shown in Table 4. The contents of these phytochemicals differed significantly between the two samples. Cakes were found to have considerably greater ($p < 0.05$) phenolic component concentrations than juice. Total polyphenols > tannins > flavonoids > anthocyanins were the most abundant phenolic components in the cakes, whereas total polyphenols > flavonoids > tannins > anthocyanins were the most abundant in the juice.

In-vitro antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity data are shown in Figure 2 and are reported in terms of inhibitory Concentration 50. (IC50). The lower the IC50 value, the greater the antioxidant activity of the investigated fruits powder. Cakes had the highest antioxidant activity in fruit extracts (IC50: 66.17 µg/mL), followed by juice (IC50: 386.54 µg/mL). Moreover, a significant difference was observed ($p > 0.05$) when compared to the Vit C group used as the reference group, ascorbic acid (IC50: 34.00 µg/mL).

Table 4. Bioactive compounds content of *C. edulis* juices and cakes

Compounds	Value	
	Juice	**Cake**
Polyphenols, mg GAE/g DW	14.10 ± 0.65	16.12 ± 0.63*
Flavonoids, mg/g DW	4.65 ± 0.16	5.02 ± 0.05*
Tannins, mg GAE/g DW	2.56 ± 1.14	5.97 ± 0.19**
Anthocyanins, mg/g DW	0.20 ± 0.02	2.13 ± 0.02***

Values were expressed as means ± SD (n = 3). Values from the cake group were statistically compared against the juice group. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) using the Duncan's multiple comparison test.

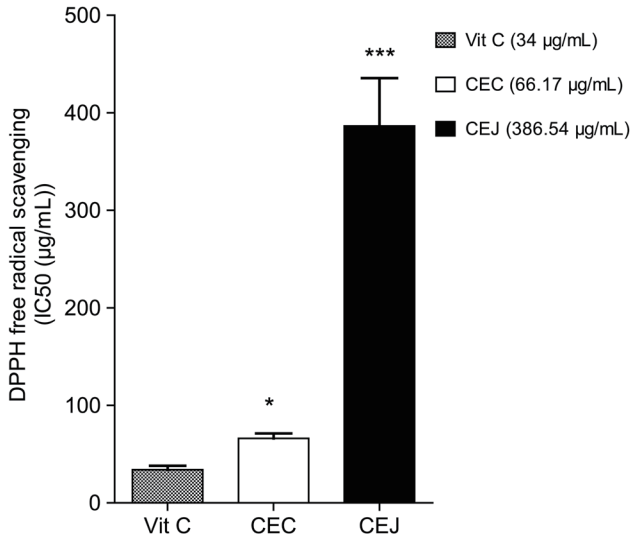

Fig. 2. DPPH free radical scavenging of fruits juices and cakes of *C. edulis*. Data were expressed as means ± SD (n = 3) and compared using the Duncan's multiple comparison test. Significance was indicated in comparison with the Vitamin C (Vit C) group. (*$p < 0.05$ for CEC, Carissa edulis cake; ***$p < 0.001$ for CEJ, Carissa edulis juice).

Total reducing power

The reducing power test is a simple and quick screening approach for determining antioxidant potential. The potassium ferric cyanide reduction determines the color shift of the test solution from yellow to different shades of green and blue based on the reducing power of each sample. The overall reducing power of the juice and cake made from *C. edulis* fruits is shown in Figure 3. When compared to juice (54.13 mg EAA/g DW), the cake a significantly higher reducing power (80.45 mg EAA/g DW).

Total antioxidant capacity

The phosphomolybdate test assesses a sample's capacity to eliminate a free radical by transferring an electron to it. Figure 4 depicts the overall antioxidant capacity values of the cake and juice. The phosphomolybdate technique works by reducing Mo (VI) to Mo. (V). The antioxidant sample is identified by the production of a green phosphomolybdenum hue (V). The data show that the cake (111.00 mg EAA/g DW) had the highest overall antioxidant capacity, followed by the juice (103.92 mg EAA/g DW), with significant differences ($p < 0.05$).

Discussion

This study aimed to assess the nutritional value, phenolic content, and antioxidant properties of cake and juice made from *Carissa edulis* fruit. It appears from Table 1 that the fruits of *C. edulis* were juicy, and their measured juice contents were 60.64 ± 1.59%. Some previous studies on other wild fruits reported a close value of juice content such as pomegranate (50.25–64.17%);[27] or *Purni-*

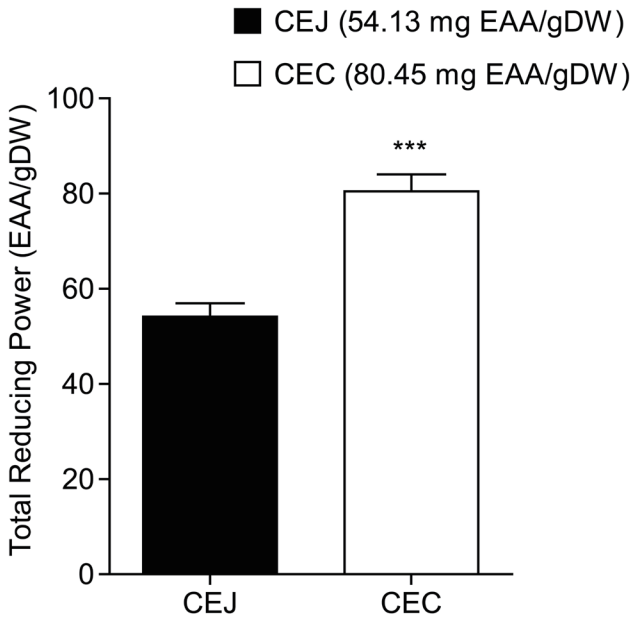

Fig. 3. Total reducing power of juice and cake of the fruits of *C. edulis*. Data were expressed as means ± SD (n = 3) and compared using the Duncan's multiple comparison test. Significance was indicated compared to the Carissa edulis juice (CEJ) group. (***$p < 0.05$ for Carissa edulis cake CEC).

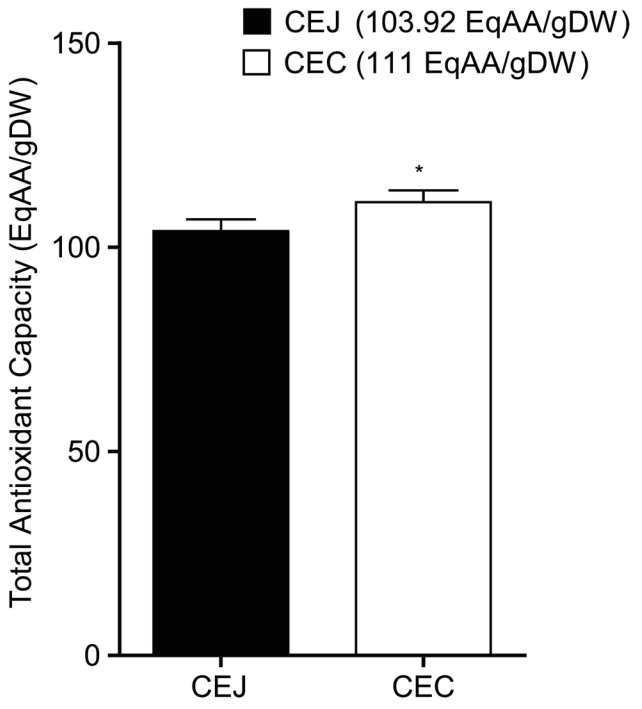

Fig. 4. Total antioxidant capacity of the juices and cakes of the fruits of *C. edulis*. Data were expressed as means ± SD (n = 3) and significance was determined compared to the Carissa edulis juice (CEJ) group. (*$p < 0.05$ for the Carissa edulis cake (CEC)) using the Duncan's multiple comparison test.

ma granatum (35.4–74.3%).[28] The values obtained were twice higher than those reported in conventional fruits like pineapple (36%);[29] black cherry (31.6%),[30] and cherry (30%).[31] However, they were lower than those found by Dossou *et al*. (2019)[32] with *Anacardium occidentale* (56.77–69.46%). The extraction yield varied between 40 and 50.8% fresh fruits in *Citrus reticulata* fruits.[33] The reported extraction yield of 23–49% fresh fruits on 72 varieties of *Citrus satsuma* and *Citrus reticulate* fruits.[34] The variability of juice extraction content is generally linked to certain parameters such as the extraction process used, the type of press, the maturity of fruits, their water content, etc.[35] The high juice extraction yields observed on the fruits studied suggest industrial production of these juices. Seeds represent 13.90 g/100 g of fruits of the mass of *C. edulis* fruits, the results confirm that these fruits are as highly juicy as mangoes (78.9%), apples (84.2%), or grapes (81.6%).[36] Dry matter, which refers to the material that remains after the removal of water, is an indication of the number of nutrients accessible to the organism in a given meal.[37] The cake of these fruits has higher dry matter content (64.14 g/100 g fresh cake weight) than those of their juices (09.28 g/100 g fresh cake weight). This observation is normal because the juices have higher water content. The cake obtained after extraction of juice can also be revalorized in animal feed or other food formulations according to its nutritional and biological properties such as antioxidant activity.

Moisture levels ranged from 5.67% for cake powder to 14.40% for juice powder on a wet basis. This result is equivalent to the moisture content of previously reported Bus mango cake (8.4%), Pumpkin blended cake (6.01%), and *Phoenix dactylifera* cake (6.07%).[38,39] Powder stability, storage qualities, and other technical features are affected by the humidity content and water activity of powder samples.[40] It is well known that a moisture level less than 14% ensures powder stability during storage, whereas greater moisture content greater promotes the growth of microorganisms and product degradation.[41] The increased moisture content of juice and cake powder might be attributed to their hygroscopic properties. The capacity of materials to absorb moisture in the environment is referred to as hygroscopicity. According to some authors, the hygroscopic index of a powder is acceptable when lower than 20 g/100 g DW.[42] Furthermore, the presence of protein might be connected to the high moisture content of powder, because protein has a greater water-holding capability inside its amorphous state.[43]

Fat contents are presented in Table 2. The cake powder included substantially ($p < 0.05$) more fats (22.68%) than the juice (5.06%). These results are comparable to recent studies on *Phoenix dactylifera* cake (5.37%),[39] and *Mangifera indica* juice (25.57%).[44] These findings imply that fruit cakes might be a good source of vegetable oil. In this investigation, the fat contents of *Mangifera indica* (0.7%)[38] and *Citrus maxima* juices (0.83%) were considerably ($p < 0.05$) greater than those of other typical fruits juices (0.83%).[45] As previously demonstrated, the low-fat level of fruit juice makes it an appropriate component of weight-loss programs.[45]

The ash contents in juice powders were 0.31% and were 0.28% for the cakes. Ash is a good predictor of the mineral concentration in a sample. From a comparative point of view, our results are similar to those obtained previously on lyophilized *C. edulis* pulps (0.21%),[46] and the *Anacardium occidentale* fruits (0.25%).[47] However, the ash value found here was lower compared to previous values found on the *Citrus maxima* juice (0.7%),[45] and the *Rhus coriaria* fruits pulps (2.87%).[48]

The carbohydrates contents were ranged from 19.29 g/100 g DW for the juice to 39.25 g/100 g DW for the cake. Carbohydrate was the most abundant macronutrient as in the majority of fruits. This conforms with other studies[45,49,50] which found higher

carbohydrates contents in citrus pulps. The carbohydrates content obtained in our study is higher than those obtained on *Carissa macrocarpa* pulps (21.57 g/100 g DW).[51] Compared with juices of conventional fruits, our results are 1.5 times as high as those obtained on *Citrus maxima* fruit juice (16.79 g/100 g DW),[45] and three times higher than those obtained on *Anacardium occidentale* fruits juice (9.9 g/100 g DW).[52] Juice and cakes of *C. edulis* fruits might be considered as a source of carbohydrates particularly in animal feed production. Carbohydrate is energy-producing food that provides readily available fuel for physical and other bodily activities.

The juice and cake of the studied fruit contain low protein. Protein contents of juice powders were lowest with the values 0.23, while protein contents of the cake powders were 1.32 g/100 g DW. The diversity in protein concentration might be attributable to the diverse varieties of fruits utilized, most likely owing to the fruits' varying nitrogen-containing components. The protein content reported in this study coincides with the values obtained with the *Mangifera* juice (1.1 g/100 g DW),[38] *Citrus maxima* juice (1.76 g/100 g DW),[45] and *H. barteri* pulps[53] (1.5 g/100 g DW) and by Amouzou *et al*., 2013 (0.813 g/100 g DW).

Carotenoids are the primary pigments of plants that give them distinctive hues such as yellow and orange.[54] Total carotenoid concentration in the studied fruits was substantially ($p < 0.05$) higher in juice extract than in cakes. Also, total carotenoid content was dramatically lower within the cake than in the juice, as indicated in Table 3. These variations might be connected to the color of the peels of the fruit. Carotenoids concentrations in this study were similar to that found in orange juice (0.81 mg/100 g DW)[54] but much lower than the carotenoid content of other fruits such as pomegranate juice (23 mg/100 g DW) observed in the same study.[54] However, the carotenoid levels found in this study were significantly greater than that found in *Anacardium occidentale* fruits (0.39 mg/100 g DW).[55]

Vitamin C is an antioxidant compound that neutralizes the effects of free radicals and prevents diseases. The high Vitamin C content of the juice compared with the cake powder may be due to their hydro-solubility character. A similar observation was reported on the Vitamin C content between juice (26.36 mg/100 g DW) and peel (19.34 mg/100 g DW) of *Citrus maxima*.[54] The concentration of Vitamin C found in our samples was lower compared to those found in some conventional fruits such as strawberry juice (49 mg/100 g DW).[56] The quantity of Vitamin C detected in this study was higher than that found in the orange (18.9 mg/100 g DW)[57] and Cherry juices (16 mg/100 g DW).[58] These authors did, however, observe greater Vitamin C concentration in *Anacardium occidentale* (86.22 mg/100 g DW) and *Mangifera indica* (67 mg/100 g DW) juices.

Minerals are essential for appropriate nutrition and metabolism, and their importance cannot be overstated. Many variables influence the mineral content of fruits, including soil type, maturity stage, cultivar variety, terrain, and other geographical considerations. Iron is the third most abundant mineral in our samples study after calcium and copper. As shown in the table of mineral content, the iron content of juice was 1.34 mg/100 g DW vs 1.47 mg/100 g DW for cake. There was no notable change ($p < 0.05$) between the two samples. Other research on wild fruits discovered comparable results with *Prunus domestica* (1.08 mg/100 g DW),[55] and *Prickly pear* juices (1.36 mg/100 g DW).[45] In comparison, the iron value measured here has been higher than that published on other traditional fruits like dates (0.9 mg/100 g DW).[39] Iron is a trace metal that functions as a cofactor of catalase.[59] It also participates in the formation of hemoglobin by combining with porphyrin to generate heme.[60]

Zinc is an important micronutrient that protects the body against oxidative stress and stimulates immune mechanisms.[60] The cake of *C. edulis* fruit presented a higher content in this mineral as compared to the juice (Table 3). Significantly ($p < 0.05$) higher zinc concentrations were noted in *C. edulis* cake (4.28 mg/100 g DW). The *C. edulis* juice (1.88 mg/100 g DW) had the lowest concentration of this element (1.88 mg/100 g DW). The zinc value obtained was comparable to those described by Amouzou *et al*. (2006) on *H. barteri* pulps (2.2 mg/100 g DW)[61] but lower than previously discovered by Liu *et al*. (2020) (14 mg/100 g DW) on the same pulps.[60] The result found here are ten times more than those obtained by earlier authors on typical fruits such as P*runus domestica* fruits (0.2 mg/100 g DW)[60] or the date spam fruits juices (0.21 mg/100 g DW).[39]

The copper levels in the cake (1.12 ± 0.84 mg/100 g DW) were significantly ($p < 0.05$) greater than in the juice powders (0.44 ± mg/100 g DW). Copper concentrations values consistent with those published in the apple juice (0.58–0.76 mg/100 g DW)[62] and pulps of *H. barteri* fruits (0.7 mg/100 g DW).[11] However, they are ten times higher than those found in *Phoenix dactylifera* fruit juice (0.07 mg/100 g DW).[39] Copper is a cofactor of superoxide dismutase, and hence has a function in erythropoietic production.[63] As a result, the *C. edulis* fruit cake may be effective as an antianemic or antioxidant agent.

Calcium was the first most abundant mineral identified in the cake and juice of the tested fruits and its content was significantly ($p < 0.05$) greater in the cake powders. The values ranged from 43.78 ± mg/100 g DW for the juice to 80.52 ± mg/100 g DW for the cake. These results are in close line with those found in the *H. barteri* (80 mg/100 g DW),[61] and *Mangifera indica* pulps (40–49 mg/100 g DW),[64] and the *Prickly pear* juice (83–89 mg/100 g DW).[65] Furthermore, they were much greater than those obtained with *Prunus domestica* (12 mg/100 g DW),[66] and *Anacardium occidentale* (12 mg/100 g DW). Calcium is essential in many biological functions (cardiac automatism, in the contraction of smooth and striated muscles, nerve conduction, coagulation, and endocrine and exocrine hormonal secretions).[67] This calcium decreases the bioavailability of dietary iron by competition at the site of absorption and therefore can lead to iron-deficiency anemia.[68]

Phytochemicals are important bioactive molecules that have been linked to a variety of health benefits. Polyphenols are abundant in the cake extract, trailed by tannin, flavonoids, and anthocyanins. These antioxidants were also found in significant quantities in the juice.

It should be noted that the concentration of polyphenolic compounds varied significantly between liquid fruits versus cake powders made from the same fruits. Fruit cake powder (16.12 ± 0.63 mg GAE/g DW) had a considerably ($p < 0.05$) greater total phenolic content than juice powder (14.10 ± 0.65 mg GAE/g DW). Similar findings were observed by previous authors on *C. edulis* fruit pulps[69] (9.54 mg GAE/g DW) and 27 citrus cultivars (2.6–10.45 mg GAE/g DW).[70] Our results also outperform those of orange juice (3.29 mg GAE/g DW),[57] *Citrus maxima* juice (1.8 mg GAE/g DW),[45] and pomegranate juice (5–8 mg GAE/g DW).[50] These findings indicate that *C. edulis* juices and cakes have vital biological qualities, as evidenced by multiple recent studies demonstrating the advantages of these secondary metabolites.[71]

The highest contents of flavonoids were observed in the cake compared to the juice. Flavonoid content ranged from 4.65 mg RE/g DW for the juice to 5.02 mg RE/g DW for cake powder of *C. edulis* fruits. The results of this study are comparable with the quantities found in the juice of *Citrus maxima*.[45] However, these flavonoid contents are four times lower than those obtained in the cake of *Citrus* fruits (16.42 mg RE/g DW),[70] and the juice of *pome-*

granate fruits.[50] Flavonoids are an important group of phenolic compounds in plants with a wide range of biological properties such as antioxidant, anti-inflammatory, anticancer hypoglycaemic, and hypocholesterolemic activities.[72]

In addition to polyphenols and flavonoids, the greatest ($p < 0.05$) tannin concentration (Table 4) was found in the cake powders when compared to the juice powders. In this analysis, the cake sample had a higher ($p < 0.05$) tannin concentration (5.97 ± 0.19 mg GAE/g DW). The tannin level in juice samples was lower (2.56 ± 1.14 mg GAE/g DW). The tannin level of the juice is commensurate with the tannin content of *H. barteri* fruit pulp (2.13 mg GAE/g DW),[73] and the *Anacardium occidentale* juice (2.05–6.6 mg GAE/g DW).[32] Besides, *pomegranate* juice has a greater tannin concentration (6.6 mg GAE/g DW).[50]

Anthocyanins levels of powder samples are presented in Table 4 The value of these beneficial substances ranged from 0.20 mg/g DW to 2.13 mg/g DW for the juice and cake of *C. edulis* fruits, with significant variations ($p < 0.05$). These results are comparable to the Anthocyanin contents of blackberry juice (0.7–1.34 mg/g DW)[74] and pomegranate juice (0.9–1 mg/g DW) reported previously.[50] Anthocyanins are the chemical substances that give the juices and fruits of *H. barteri* and *C. edulis* their purplish-red color.[75] However, the current study results are 10 times higher than those reported in *Anacardium occidentale* fruits juice.[55]

According to the results, as compared to the contents of bioactive components in the cake, tested juice samples contained lower amounts of phenolic compounds as compared to the fruit's cake and considering that most of these compounds remained in the pomace during the typical extraction technique.[76,77]

The cake had the highest antioxidant activity (lowest IC50) in the DPPH experiment when compared to the juice. This shows that phenolic compounds had a substantial role in the antioxidant activity of the fruit powders studied. This is consistent with the fact that plant products' antioxidant activity is often attributed to the radical scavenging activity of phenolic components such as flavonoids, polyphenols, and tannins.[58,78] The antioxidant activity of phenolic compounds is mostly owing to their redox characteristics, which can be useful in scavenging free radicals, quenching singlet and triplet oxygen, and degrading peroxides.[79] Indeed, phenolic compounds with multi hydroxyl (OH) group moieties and unsaturation centers can promote DPPH neutralization.[80] The high bioactive content of the examined fruits powders is most likely responsible for their strong antioxidant action. Previous studies have demonstrated the *in vitro* antioxidant properties of various solvent extracts obtained from *C. edulis* fruits. For instance, Fowsiya and Madhumitha (2017) found that the ethanol extract of *C. edulis* exhibited the best DPPH scavenging activities compared to the water, petroleum, ethyl acetate, chloroform extracts respectively.[81] Likewise, Ojerinde *et al*. (2021) revealed that the methanol extract of fruits displayed strong antioxidant activities with the following efficient concentrations (DPPH, IC50 = 87.98 µg/ml; FRAP, EC50 = 464.33 µg/ml & Ferrous chelating, EC50 = 294.55 µg/ml).[82] In addition, Woode *et al*. (2008) showed a lower antioxidant activity of the ethanolic extract of roots compared to fruits (IC50: 210 vs 71 µg/mL).[83] On contrary to these studies, Fanta *et al*. (2019) showed that leaves aqueous extracts displayed stronger DPPH scavenging effects compared to organic extracts with an IC50 of 0.304 µg/mL.[84]

Because the presence of antioxidants in the fruit juice and cake induces the conversion of the Ferric cyanide complex into ferrous form, the ferrous iron complex may be measured by monitoring the production of Perl's Prussian blue powder at 700 nm. The higher the absorbance value, the greater the reduction power of the samples (Fig. 3). In this investigation, fruit cakes outperformed juice in terms of lowering power, with a statistically significant difference ($p < 0.05$). The antioxidant potential of the fruits studied is determined by their phenolic content. Previous research has found a link between decreasing power and plants' phenolic chemicals.[85] According to other studies, substances with reducing power can diminish the oxidized intermediates of lipid peroxidation processes.[86,87]

The TAC is an *in vitro* test that gives an overall estimate of the antioxidant power of all the bioactive compounds (phenolic compounds, vitamins) present in a given sample. It can either use a single-electron transfer method monitored spectrophotometrically by a color change, or a hydrogen atom transfer reaction measured by the elimination of peroxyl radicals.[88] In the present study, the obtained findings showed that cakes had a better TAC compared to juice which is consistent with the previous effects observed on DPPH and the ferric reducing power. These results tend to prove that the concurrent presence of bioactive ingredients in a sample could be seen as a factor that boosts biological activity. Indeed, Patil *et al*. (2016) demonstrated that piperine which is a natural alkaloid compound found in pepper could increase the bioavailability of curcumin (flavonoid compound) when mixed with it and this, through intercalation mechanisms including the intermolecular bonding formation.[89] Also, Duan *et al*. (2004) found that polyphenolic compounds contained in the ethyl acetate extract of *Galla chinese* might separately exert strong inhibitory activity on the hepatitis C virus protease conferring thereby strong antiviral activity to this extract.[90]

Future studies

The findings of the present study tend to highlight the *in vitro* antioxidant potential of the *C. edulis* cakes compared to juice. Further investigations on the *in vivo* antioxidant properties are required to understand how it can interfere or regulate the reactive oxygen species production and the activity of endogenous antioxidants including glutathione, antioxidant enzymes, at the protein and gene levels in a living organism Also, the contribution of *C. edulis* cakes to the management of stress-related diseases like anemia is not to be ruled out. As the cakes were found to contain a high number of secondary metabolites including carotenoids, anthocyanins, tannins, it would be interesting to analyze the impact of these compounds on the bioavailability of the cakes and how it could affect the antioxidant potential *in vivo*.

Conclusions

This study aimed to determine the nutritional value, the contents in bioactive compounds, and the antioxidant properties of the juice and cake from *C. edulis* fruits harvested at the dark red stage. It can be concluded that the cakes possessed higher macro and micronutrients contents, phenolic compounds, and higher antioxidant activity than juices. Thus, the evaluation of the nutritional value and antioxidant of *C. edulis* fruits indicate that it is important for food supplementation or diet diversification and is useful to promote the cultivation of suitable fruits accessions as a source of phytochemicals and vitamins for a supplement to diet and potential for added value.

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Conflict of interest

The authors have no conflicts of interest to disclose.

Author contributions

Study design (DK, NYN), the performance of experiments (DK, ANA), analysis and interpretation of data (DK, BRTG, NYN), manuscript writing (DK, BG, NYN) critical revision (DK, BRTG, MD, ANA, NYN, AB), statistical analysis (DK, NYN), study supervision (NYN, AB), and technical support (BRTG).

Data sharing statement

Data of this study can be obtained upon request to the authors.

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