



Chemical and Sensory Properties of Zobo (*Hibiscus sabdariffa calyces*) Drink Fortified with *Justicia carnea* Leaf Extracts

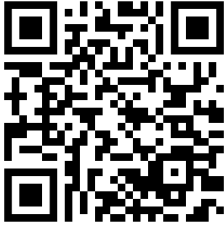

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Abstract	Article History
<p>This study evaluated the chemical and sensory properties of zobo drink fortified with <i>Justicia carnea</i> leaf extracts was evaluated. Zobo drink was produced from <i>Hibiscus sabdariffa calyces</i>, and used as the control (sample A, 100% zobo drink), and <i>Justicia carnea</i> extract was also produced and used as blends with the zobo drink at varying levels of inclusion- 36.57% (sample B), 42.31% (sample C), 44.61% (sample D), 57.69% (sample E) and 54.15% (sample E) using the Pearson square formulation ratio. Physicochemical properties, Proximate, Mineral content, Iron bio-accessibility, Phytochemical properties and Sensory evaluation were carried out on the control, as well as the blends. Moisture content of the control and the blends ranged from 77.82 to 84.43% with the control having the highest value. The inclusion of <i>Justicia carnea</i> extract to zobo drink reduced the fat content of the drink, as the control (100% zobo drink) recorded fat content of 0.27%, and reduced after blending with <i>Justicia carnea</i> extract, ranging from 0.04-0.16%. There was no significant difference ($p < 0.05$) in the ash content of the samples which ranged from 0.55-0.60%. The Total Soluble Solid of the samples ranged from 14.85-16.75 °Brix. Blending <i>Justicia carnea</i> extract with zobo drink increased the Total Solid (TS) and pH of the blends which ranged from 17.34-22.18 mg/L and 3.12-3.28 respectively. The mineral content ranged from 19.00-23.52 mg/100g (for Magnesium), 0.07-0.92 mg/100g (for Zinc) and 2.32-7.77 mg/100g (for Iron). Result showed that the inclusion of <i>Justicia carnea</i> extract increased the iron content of the drink. Iron bio-accessibility result indicated that the accessibility of the iron content of 100% zobo drink was 28.88%, while those of the blends ranged from 30.98-39.45%. Phytochemical properties result showed that the flavonoid content ranged from 13.82-17.43%, alkaloid content ranged from 1.23-1.95%. The blends were more acceptable in terms of taste (7.20-8.50) and flavour (6.60-7.67). Overall acceptability score showed that sample C (7.80) was rated better than the other samples. There was however no significant difference ($p > 0.05$) in the overall acceptability of the control and the blends, as well as the flavour, colour and consistency.</p> <p>Keywords: <i>Justicia carnea</i> extract, Iron bio-accessibility, Zobo drink, Fortification</p>	<p>Received: 13 Jun 2024 Accepted: 05 Jul 2024 Published: 27 Dec 2024</p> <div style="text-align: center;">  <p>Scan QR code to view*</p> </div> <p>License: CC BY 4.0*</p> <div style="text-align: center;">  <p>Open Access article.</p> </div>
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Introduction

Nigeria, like many other countries, has recognized the importance of addressing iron deficiency anemia and has implemented strategies to combat it. One of the methods used is iron fortification, which involves adding iron to commonly consumed foods. In Nigeria, this often includes the

fortification of staple foods such as wheat flour and edible oil (Abioye *et al.*, 2017). This practice has been promoted as a way to increase iron intake, especially in regions where dietary iron sources are limited. In addition to iron fortification, Nigeria, like many other countries, has a rich tradition of using natural means, including herbs and traditional foods, to

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improve nutritional content and address health issues (Oboh *et al.*, 2012). Some herbs and plants in Nigeria are known for their iron-rich properties, and they are often incorporated into traditional diets. Examples include moringa, baobab fruit, bitter leaf, sorghum, uguwu (pumpkin leaves) etc. (Abdullahi *et al.*, 2015). Combining these natural sources with iron-fortified foods and supplements, when necessary, can be an effective approach to combat iron deficiency anemia in Nigeria (Ijarotimi *et al.*, 2013).

Zobo drink, a traditional Nigerian beverage made from zobo calyx, scientifically known as *Hibiscus sabdariffa calyces*, is a popular and culturally significant beverage known for its tart flavor and deep red color. It is a popular herbaceous plant that is widely cultivated and used for various purposes. It is native to West Africa and is commonly grown in countries such as Nigeria, Sudan, and Senegal (Mohammed, 2021). According to Olayemi *et al.* (2011), there are three types of *Hibiscus sabdariffa calyces* used in zobo drink preparation which are dark red, bright red, and wine-coloured varieties. The calyx of the zobo plant has been utilized for their medicinal, nutritional, and culinary properties for centuries. Rich in antioxidants, vitamins, and minerals, zobo drink provides an excellent platform for fortification with iron-rich plant extracts (Owokotomo and Akinoso, 2016; Yusuf, 2022). One of the most popular uses of zobo calyx is in the production of a refreshing drink called zobo or sorrel drink. The calyces of the zobo plant are boiled to extract a deep red-colored liquid, which is then sweetened and sometimes flavored with spices or fruits. Zobo drink has gained popularity overtime due to its high nutritional value but the iron content does not meet the required daily allowance of iron for children and adults (Adeniji, 2017).

Justicia carnea, is a perennial shrub native to tropical and subtropical regions, known as a versatile plant with various applications (Islam *et al.* 2011). One of the significant components of the plant is the leaf, which has gained considerable attention due to its potential in diverse fields such as medicine, biofuel production, and agriculture (Dehgan, 2012). *Justicia carnea* is a plant species indigenous to various parts of the world, including Africa. It is widely recognized for its medicinal properties and has been traditionally used to treat various ailments. Various studies have identified several medicinal properties associated with *Justicia carnea* leaves, including analgesic, anti-inflammatory, antimicrobial, antifungal, and antiviral activities (Sharma *et al.*, 2021; Ahmad *et al.*, 2022; Gupta *et al.*, 2023). These properties make it a valuable resource for treating a wide range of health conditions, such as wounds, skin infections, respiratory ailments, and gastrointestinal disorders. Studies have also shown that *Justicia carnea* leaves are a potential source of iron, making them a promising tool for fortifying food and beverages (Oguntunde *et al.*, 2019; Cakmak and Kutman, 2018). *Justicia carnea* leaf extracts as a source of iron and its bioavailability when incorporated into food products (Adaramola *et al.*, 2018). It may be important in the enrichment of zobo drink with iron, which can help combat iron deficiency anemia effectively. The fortification of zobo drink with *Justicia carnea* leaf extracts will enhance the iron content of the beverage, making it a convenient and accessible

source of iron for vulnerable populations (Owokotomo and Akinoso, 2016).

Materials and Methods

Sample collection

The dark red colour Zobo calyx (*Hibiscus sabdariffa* C.) was bought from Mile 3 Market, Port Harcourt, Rivers State, Nigeria while *Justicia carnea* leaves were harvested from the Rivers State University School Farm, Port Harcourt City Local Government Area, Rivers State.

Extraction of *Justicia carnea* leaf extract

Fresh *Justicia carnea* leaves were harvested and manually sorted to remove dirt and strenuous materials. Two hundred and twenty grams (220 g) of the leaves were washed in running water and then boiled in 3700 ml of water for 30 mins at 100°C to get the extract which was filtered with a muslin cloth and stored for further use.

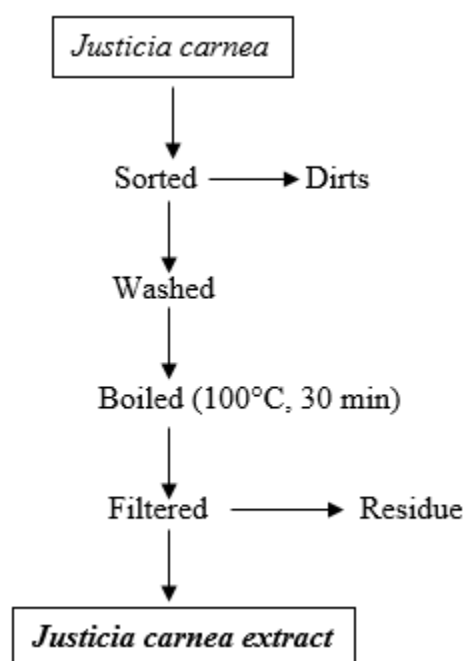


Figure 1: Extraction of *Justicia carnea* leaf extract

Zobo drink preparation

Dried hibiscus calyx (zobo) was rinsed thoroughly with running water to remove any dirt or impurities. Three hundred grams (300 g) of the leaves were be boiled in 1600 ml of water over medium heat for 15 minutes. Sixteen grams (16 g) of ginger were washed, cleaned and blended, and poured into the boiling zobo calyx while 48 g of pineapple peels (the pineapple was washed in running water and then peeled with a kitchen knife) were added to the boiling zobo calyx to give flavor and aroma to it. After boiling, the pot was removed from the heat and 150 g of sugar was added, and the mixture allowed to cool to room temperature, and stored in plastic bottles pending further use

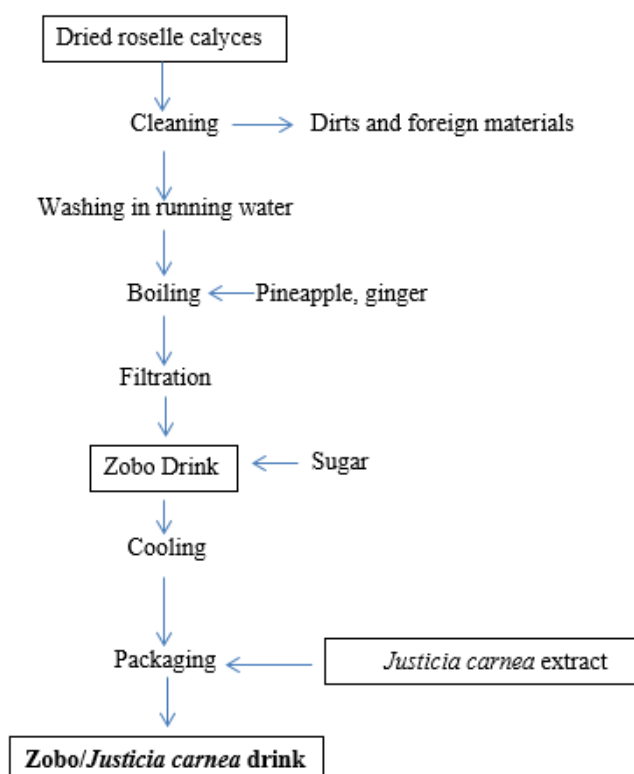


Figure 2: Preparation of zobo drink fortified with *Justicia carnea* extract.

Source: Braide *et al.* (2012) with modification

Formulation of zobo drink/*Justicia carnea* extract blends

The iron content of the zobo drink, and the *Justicia carnea* extract were determined using AOAC (2012). Pearson square was used for the formulation of the blends, as described by (Wagner and Stanton, 2012). The combination of the zobo drink and *Justicia carnea* blends extract was done based on the required daily allowance for iron (7-15 mg) (Kumar *et al.*, 2022). The blends were formulated from 1600 ml of zobo drink/*Justicia carnea* extract, and packaged in airtight containers, labeled and preserved in the refrigerator (4°C) until needed for analyses.

Table 1: Formulation of Zobo drink/*Justicia carnea* extract blends

SAMPLES	A	B	C	D	E	F
Zobo drink (ml)	1600	1014.88	923.04	886.24	676.96	733.60
<i>Justicia carnea</i> leaf extract (ml)	-	585.12	676.96	713.76	923.04	866.40
Total	1600	1600	1600	1600	1600	1600

Keys:

A= 100% zobo drink (Control)

B= 63.43% zobo drink, 36.57% *Justicia carnea* extract

C= 57.69% zobo drink, 42.31% *Justicia carnea* extract

D= 55.39% zobo drink, 44.61% *Justicia carnea* extract

E= 42.31% zobo drink, 57.69% *Justicia carnea* extract

F= 45.85% zobo drink, 54.15% *Justicia carnea* extract

Physicochemical analysis

The pH, Sugar (°Brix) and total solid (TA) was determine using standard method described by Association of Official Analytical Chemists (AOAC, 2012).

pH determination

The pH of the juice was determined using a digital pH meter (pHs-2F, Harris, England). Exactly 50 ml of the juice was transferred into a beaker and the pH was determined after the meter was calibrated using standard buffer solutions of pH 4.0 and 7.0. Sufficient time was allowed for equilibration before readings were taken.

Sugar content (°Brix)

The hand-held sugar refractometer was used. The prism of the refractometer was cleaned and a drop of the juice was placed on the prism and closed. The total sugar content (°Brix) was read off the scale of the refractometer when held close to the eye.

Total solid (TS)

For the estimation of TS, 10 ml of the sample was evaporated in an oven at 105°C and the solids left after evaporation were weighed and values for TS was calculated.

Determination of nutrient composition and energy value

The moisture, protein, fat and ash contents of samples were analysed using the standard analytical method described by AOAC (2012). Moisture was obtained gravimetrically after drying to a constant weight at 70°C in a hot air oven (DHG 9140A). Fat was determined using soxhlet extraction method with petroleum benzene. Kjeldahl method and a nitrogen conversion factor of 6.25 was used for crude protein determination. Ash content was determined gravimetrically after the incineration of the samples in a muffle Furnace (Model SXL) at 550°C for 2 h. The total carbohydrate content of the samples was determined using the Clegg Anthrone method as described by Onwuka (2018). Energy values were obtained using Atwater factor of 4 Kcal/g for protein and carbohydrate and 9 Kcal/g for fat.

Determination of mineral content

As described by Adelekan *et al.* (2014), 1 g of each sample was digested with 10% HNO₃ after ashing. The sample was filtered after digestion and the filtrate was made up to 100 mL of distilled deionized water. Atomic absorption spectrophotometer (Buck scientific, model 210) was used to determine the Magnesium, Zinc, potassium, sodium, calcium, iodine, and iron content of the samples.

Iron bio-accessibility determination

Iron bio-accessibility of samples was measured by an In-vitro method involving gastric simulations as described by Luten *et al.* (1996).

Determination of phytochemical properties

Flavonoid and alkaloid were determined by Alkaline Precipitation Gravimetric, while total phenol and tannin contents were determined by the Folin-Coicalteu method and Folin Denis Colorimetric methods respectively as described by Nbaeyi-Nwaoha and Onwuka (2014).

Sensory evaluation

Twenty-member semi-trained panelists of the Department of Home Science and Management, Rivers State University were

selected for sensory evaluation. The samples were evaluated for taste, colour, flavour and mouthfeel. Each attribute was rated on a 9-point hedonic scale of 1 to 9, with 1 representing disliked extremely, 2 representing disliked very much, 3 representing disliked, 4 representing disliked mildly, 5 representing neither liked nor disliked, 7 representing liked, 8 representing liked very much, 9 representing liked extremely as described by (Iwe, 2010).

Statistical analysis

All the analysis were carried out in duplicate. Statistical analysis was performed using Statistical Package for Service Solution (SPSS), version 26. Data obtained were subjected to Analysis of Variance (ANOVA) and difference between means were compared using Turkey's Multiple comparison tests with 95% confidence level.

Results and Discussion

Physicochemical properties of Zobo/*Justicia carnea* blends

The result of the physicochemical properties of zobo/*Justicia carnea* blends is shown in Table 2.

The sugar content of 100% zobo was 14.95 °Brix, while Zobo produced with blends of *Justicia carnea* extracts ranged from 14.10-16.75 °Brix. Sample D recorded the least (14.10 °Brix) while sample E had the highest (16.75 °Brix) sugar content. Sample E with the highest ratio of *Justicia carnea* inclusion

(57.69%) recorded the highest sugar (16.75 °Brix). Omemu *et al.* (2006) reported 11.2–13.3 °Brix after the addition of varied amount of sugar. The values from this study (14.10-16.75 °Brix) contradict Fasoyiro *et al.* (2005) who reported that roselle drink has a soluble solid of 3.20 °Brix.

The Total Solid (TS) of the zobo produced with blends of *Justicia carnea* extract ranged from 17.34-22.18 mg/L, with sample F having the least value and sample C recording the highest TS value. The control (100% zobo drink) recorded TS of 15.58 mg/L. The inclusion of *Justicia carnea* extract to zobo drink showed to increase the Total Solid (TS) of the drinks. Total solid comprise of dissolved solids plus suspended and settleable solids such as starch, sugars etc. in the beverage. The higher its value, the higher the ability of the beverage particles to settle when left to stand for some time (APHA, 1992).

The pH of the blends ranged from 3.12 (sample B) to 3.28 (sample F), while 100% zobo drink recorded pH value of 3.07. The pH of the samples increased upon inclusion of *Justicia carnea* extracts. Ajayi and Oyerinde (2020) reported pH of 2.70 for zobo drink, and pH of 3.3-3.6 for zobo and herbal blends. These results differ from the pH (3.07-3.28) record in this research. The low pH observed here will mean zobo is a high acidic drink (Babalola, 2000), and an acidic pH according to Ogunjobi and Ogunwolu (2010) is associated with the development of a pleasant taste.

Table 2: Physicochemical properties of Zobo/*Justicia carnea* blends

Physicochemical properties	A	B	C	D	E	F
Sugar (°Brix)	14.95 ^b ±0.07	14.85 ^b ±0.07	15.15 ^b ±0.07	14.10 ^c ±0.14	16.75 ^a ±0.07	14.95 ^b ±0.07
Total Solids (mg/L)	15.58 ^e ±0.32	19.39 ^b ±0.27	22.18 ^a ±0.03	19.06 ^{bc} ±0.31	18.35 ^c ±0.01	17.34 ^d ±0.08
pH	3.07 ^c ±0.03	3.12 ^b ±0.02	3.15 ^b ±0.01	3.16 ^b ±0.01	3.24 ^a ±0.01	3.28 ^a ±0.02

Values are means ± Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at $p < 0.05$.

Nutrient composition and energy value of Zobo/*Justicia carnea* blends

The result of the chemical composition of zobo/*Justicia carnea* leaf extract are shown in Table 3.

The moisture content of 100% zobo, and zobo produced with the blends of *Justicia carnea* extracts at varying proportion ranged from 77.82-84.43%, with sample C (57.69% zobo drink and 42.31% *Justicia carnea* extract) having the least moisture content and sample A (100% zobo drink) recording the highest. Moisture content determines the sample's water content and, indirectly, its dry matter content. Moisture level more than 14% according to Airaodion *et al.* (2019) makes substances susceptible to microbial growth, hence have a lower storage period. The moisture content of the zobo samples found in this research (77.82-84.43%) is way greater than the 14% spoiling threshold. Consumption of this high amount of moisture however can improve water intake in the body (Popkin *et al.*, 2010) and probably physiological processes. The results however did not differ much from Adelekan *et al.* (2014) who reported moisture content of 81.61-86.95% while studying the production and acceptability of fruits enhanced with zobo drink.

The protein content ranged from 0.26% (sample C) to 0.65% (sample B; 63.43% zobo drink and 36.57% *Justicia carnea* extract). The protein content recorded here (0.26-0.65%) was slightly lower than 0.73-0.91% protein content of 100% zobo and zobo fortified with pineapple and watermelon as reported by Onabanjo and Airaodion (2022). Though the protein content of the zobo drink obtained here

are low, it might still improve the protein consumption of the body when taken with other protein rich foods (Hudson *et al.*, 2020). There was significant difference ($p > 0.05$) between the control (Sample A) and samples B, D, E and F and hence increased in their protein contents compared with the control (sample A).

Fat content of the samples ranged from 0.04% (sample B) to 0.27% (sample A). Blending zobo drink with *Justicia carnea* extract showed to reduce the fat content of the drink. This could be as a result of the low-fat content of *Justicia carnea* leaf as reported by Anhwange *et al.* (2019). The samples with higher *Justicia carnea* blends (samples D, E and F) recorded fats content (0.12-0.16%) higher than samples B and C (0.04-0.06%) with higher zobo drink blends. These results showed to have significant difference ($p > 0.05$) from the control, sample A (0.27%).

Sample D (55.39% zobo drink and 44.61% *Justicia carnea* extract) recorded the least ash content (0.29%), while the highest ash content (0.60%) was recorded by samples A, C and F (45.85% zobo drink and 54.15% *Justicia carnea* extract). There was no significant difference between the control and the blends, in their ash contents. The results obtained here (0.50-0.60%) did not differ much from the values (0.56-0.79% ash) of Onabanjo and Airaodion (2022). The low ash content obtained here will mean low mineral content of the samples, as ash content give a rough estimate of the mineral content of foods (Mepba *et al.*, 2007).

The carbohydrate content ranged from 12.70-13.60%, with sample B recording the lowest, and sample F recording the highest carbohydrate content. The carbohydrate content of the 100% zobo drink (13.49%) differs from 11.07% reported by Onabanjo and Airaodion (2022) for 100% zobo drink. The carbohydrate content of the blends ranged from 12.70-13.60%, and differs from the carbohydrate contents of the blends of zobo and water melon (11.05%) and zobo and pineapple (11.07%) reported by Onabanjo and Airaodion (2022).

The energy value of the samples ranged from 53.60 kJ/100 ml (sample B) to 58.23 kJ/100 ml (sample F), and were similar to 49.18-

73.12 kJ/100 ml reported by Onabanjo and Airaodion (2022) for zobo drink fortified with various fruits. Samples B (53.60 kJ/100 ml), C (55.14 kJ/100 ml) and D (57.48 kJ/100 ml) with 36.57%, 42.31% and 44.61% of *Justicia carnea* blends respectively recorded lower energy values compared to the control (57.63 kJ/100 ml), indicating that *Justicia carnea* extract reduced the energy value of the sample. The inclusion of at least 54.15% (sample F) *Justicia carnea* extract however increased the energy value of the samples. This could be as a result of the higher carbohydrate content (13.60%) recorded by the sample. This trend was similar to the report of Onabanjo and Airaodion (2022).

Table 3: Nutrient composition and energy value of Zobo/*Justicia carnea* blends

Nutrients (%)	A	B	C	D	E	F
Moisture	84.43 ^a ±0.32	80.61 ^d ±0.27	77.82 ^e ±0.03	80.94 ^{cd} ±0.31	81.65 ^c ±0.01	82.66 ^b ±0.08
Protein	0.31 ^d ±0.01	0.65 ^a ±0.02	0.26 ^d ±0.01	0.55 ^c ±0.02	0.57 ^{bc} ±0.01	0.62 ^{ab} ±0.01
Fat	0.27 ^a ±0.01	0.04 ^e ±0.00	0.06 ^d ±0.00	0.16 ^b ±0.00	0.12 ^c ±0.00	0.15 ^b ±0.01
Ash	0.60 ^a ±0.00	0.55 ^a ±0.06	0.60 ^a ±0.01	0.58 ^a ±0.1	0.50 ^a ±0.00	0.60 ^a ±0.00
Carbohydrate	13.49 ^{ab} ±0.00	12.70 ^c ±0.04	13.39 ^{ab} ±0.05	13.46 ^{ab} ±0.01	13.25 ^b ±0.01	13.60 ^a ±0.16
Energy (kJ/100 ml)	57.63 ^b ±0.01	53.60 ^e ±0.00	55.14 ^d ±0.00	57.48 ^b ±0.00	56.36 ^c ±0.00	58.23 ^a ±0.01

Values are means ± Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at $p < 0.05$.

Mineral composition and Iron bio-accessibility of Zobo/*Justicia carnea* blends

The mineral composition and iron bio-accessibility result of zobo/*Justicia carnea* blends are shown in Table 4.

The magnesium content of the blends ranged from 15.11-23.52 mg/100g with sample E (42.31% zobo drink and 57.69% *Justicia carnea* extract) recording the least value, while sample D recorded the highest. The control (100% zobo drink) recorded magnesium content of 21.38 mg/100g. Samples E and F with the highest percentage of *Justicia carnea* extract inclusion recorded the least magnesium content (15.11-19.00 mg/100g). This indicates that the use of *Justicia carnea* extract on zobo drinks reduced the magnesium content of zobo drinks. The Zinc content of the samples varied significantly ($p < 0.05$) and ranged from 0.07 mg/100g (sample C) to 0.92 mg/100g (sample D). The 100% zobo drink (sample A) recorded zinc content of 0.83 mg/100g. Zinc is found in cells throughout the body. It helps the immune system fight off invading bacteria and viruses. Samples B and C with the least percentage of *Justicia carnea* extract (36.57 and 42.31% respectively) recorded the least zinc content (0.12 and 0.07 mg/100g respectively).

The sodium content of the zobo/*Justicia carnea* blends ranged from 19.87-24.39 mg/100g with sample E having the least, and sample D recording the highest sodium content. The sodium content (24.41 mg/100g) of the 100% zobo (sample A) was similar to Borokini *et al.* (2019), who fortified zobo drink with pineapple bark and found 24.43 mg/100g sodium. Sodium and potassium regulate the water balance in bodily tissues and have a role in the transfer of various non-electrolytes (Airaodion *et al.* 2021).

Potassium content of 100% zobo was 5.97 mg/100g, while the zobo/*Justicia carnea* blends recorded potassium values ranging from 6.01 mg/100g (sample D) to 7.11 mg/100g

(sample E). The potassium concentration in the 100% zobo drink in this study (5.97 mg/100g) was similar to 5.11 mg/100g reported by Nwankwo *et al.* (2022), but substantially higher than the 2.00 mg/100g found by Adeniji (2017). This figure is much lower than Borokini *et al.* (2019)'s figure of 28.10 mg/100g potassium.

Calcium content of the samples ranged from 2.11-2.81 mg/100g with sample B recording the least, and sample A recording the highest calcium content. This calcium content recorded here differed from 0.95 mg/100g calcium in unfortified zobo beverages reported by Borokini *et al.* (2019), but similar to 2.80 mg/100g reported by Adeniji (2017).

The Iron content (3.18-7.77 mg/100g) of the blends were significantly ($p < 0.05$) higher than the value (2.32 mg/100g) obtained for 100% zobo drink. Sample E recorded the highest iron content while sample A had the least value. Ajayi and Oyerinde (2020) reported 3.75 mg/100g iron content of 100%, and this result differs from the 2.32 mg/100g reported in this research. Results showed that the iron content of the blended samples increased as the inclusion ratio increased and ranged from 3.18-7.77 mg/100g, indicating that *Justicia carnea* extract increased the iron content of the zobo drinks. Zobo blends with at least 44.61% *Justicia carnea* extract improved the iron content of the drink. Sample E with 57.69% of *Justicia carnea* blends recorded 7.77 mg/100g of iron, and may meet the required 8mg/100g for adolescent male (Kumar *et al.*, 2022).

Iron bio accessibility of the samples ranged from 28.88% (sample A) to 39.45% (sample E). Bio-accessibility refers to the portion of a nutrient that is released from the food matrix and made available for absorption by the human body. Sample E, having the highest percentage (57.69%) of *Justicia carnea* extract recorded the highest iron accessibility (39.45%).

Table 4: Mineral composition and Iron bio-accessibility of Zobo/*Justicia carnea* blends

Minerals	A	B	C	D	E	F
Magnesium (mg/100g)	21.38 ^a ±0.01	19.05 ^{ab} ±0.01	21.48 ^a ±0.01	23.52 ^a ±2.82	15.11 ^b ±0.01	19.00 ^{ab} ±0.00
Zinc (mg/100g)	0.83 ^b ±0.01	0.12 ^e ±0.00	0.07 ^f ±0.00	0.92 ^a ±0.01	0.71 ^c ±0.01	0.63 ^d ±0.01
Sodium (mg/100g)	24.41 ^a ±0.01	24.00 ^c ±0.01	24.14 ^b ±0.01	24.39 ^a ±0.01	20.00 ^e ±0.01	19.87 ^d ±0.01
Potassium (mg/100g)	5.97 ^e ±0.01	6.11 ^c ±0.01	6.07 ^d ±0.01	6.01 ^d ±0.01	7.11 ^a ±0.01	7.09 ^b ±0.01
Calcium (mg/100g)	2.81 ^a ±0.01	2.11 ^f ±0.01	2.30 ^c ±0.01	2.74 ^b ±0.01	2.14 ^e ±0.01	2.16 ^d ±0.01
Iron (mg/100g)	2.32 ^e ±0.01	3.18 ^d ±0.01	3.33 ^c ±0.01	5.10 ^b ±0.01	7.77 ^a ±0.01	5.12 ^b ±0.01
Bio-accessible Iron (%)	28.88 ^f ±0.01	33.0 ^c ±0.01	38.44 ^b ±0.01	30.98 ^e ±0.01	31.92 ^d ±0.01	39.45 ^a ±0.02

Values are means ± Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at p<0.05.

Phytochemical properties of Zobo/*Justicia carnea* blends

The phytochemical properties of zobo/*Justicia carnea* drink blends are shown in Table 5.

The flavonoid content ranged from 13.82-17.43% with sample A recording the lowest, and sample E the highest flavonoid score. Inclusion of *Justicia carnea* extract increased the flavonoid content of the samples. Flavonoid concentrations in this study are higher than those (1.25-1.79 mg/100g) reported by Onabanjo and Airaodion (2022) for flavoured zobo drinks. The alkaloid content of the samples ranged from 1.23-1.82%. Sample D had the lowest value while sample B recorded the highest. Alkaloids have been shown to have analgesic, antispasmodic, and antibacterial effects (Airaodion and Ogbuagu, 2020a). They have also been linked to antihypertensive and detoxifying activities (Airaodion and Ogbuagu, 2020b). As a result, zobo blended with *Justicia carnea* will be more therapeutic than plain zobo.

Tannin content of the samples ranged from 6.84 mg/kg (sample E) to 13.31 mg/kg (sample A). Fortifying zobo drink

with *Justicia carnea* reduced the tannin content of the samples. Tannins cause decreased feed consumption in animals, bind dietary proteins and digestive enzymes to form complexes that are not readily digestible. Tannins can also interact with dietary iron by preventing its absorption and they have the capability of decreasing the digestibility and palatability of proteins because they form insoluble complexes with protein (Adelekan *et al.*, 2014).

The total phenol content of the samples ranged from 28.84-53.42 mg/100g with sample E recording the lowest, and sample A having the highest total phenol content. The total phenol concentration was reduced by *Justicia carnea* inclusion. Phenols have been shown to exhibit a variety of biological functions (Ghasemzadeh *et al.*, 2010), some of which include increased bile production, lower blood cholesterol and lipid levels, and antibacterial activity against bacteria such as staphylococcus aureus (Onabanjo and Airaodion, 2022).

Table 5: Phytochemical properties of Zobo/*Justicia carnea* blends

Phytochemical properties (%)	A	B	C	D	E	F
Flavonoid	13.82 ^c ±0.40	16.79 ^{ab} ±0.89	16.53 ^{ab} ±0.01	15.40 ^{bc} ±0.25	17.43 ^a ±0.61	16.47 ^{ab} ±0.30
Alkaloid	1.68 ^{ab} ±0.06	1.95 ^a ±0.01	1.73 ^{ab} ±0.01	1.23 ^c ±0.01	1.54 ^b ±0.17	1.49 ^b ±0.07
Tannin (mg/kg)	13.31 ^a ±0.02	10.89 ^b ±0.01	9.91 ^c ±0.01	9.11 ^d ±0.01	6.84 ^f ±0.01	7.74 ^e ±0.04
Total phenol (mg/100g)	53.42 ^a ±0.01	47.76 ^b ±0.04	42.04 ^c ±0.14	38.74 ^d ±0.52	28.84 ^f ±0.07	36.25 ^e ±0.40

Values are means ± Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at p<0.05

Sensory evaluation of Zobo/*Justicia carnea* blends

Sensory evaluation scores of zobo/*Justicia carnea* drink blends are shown in Table 6.

The sensory score for taste for the samples ranged from 6.20-8.50, with sample A being scored the lowest, and sample C scoring the best. The blended samples recorded the best taste scores and ranged from 7.20-8.50 as against the control (sample A) which recorded the least taste score of 6.20 and can be attributed to its low total solid score (15.58 mg/L) as suggested by Weriko-Manu *et al.* (2016).

The colour score of the samples ranged from 7.00 (sample B) to 7.90 (sample C). Adelekan *et al.* (2014) reported colour

score of 7.66 for zobo flavoured with pineapple and pepper fruit.

The flavour score ranged from 5.80 (sample A) to 7.67 (sample F). The inclusion of *Justicia carnea* extract to zobo drink improved the flavour of the drink as the flavour of the control (100% zobo) increased from 5.80 and ranged from 6.60-7.67. There was however no significant difference (p<0.05) among the control and the blends.

The mouthfeel score recorded for the samples ranged from 6.60 (sample D) to 7.90 (sample C). There was also no significant difference (p<0.05) amongst the samples in their mouthfeel scores.

The overall acceptability ranged from 6.20-7.80, with sample B recording the least, and sample C recording the highest.

There was no significant difference ($p < 0.05$) in the overall acceptability of the samples

Table 6: Sensory evaluation of Zobo/*Justicia carnea* blends

Sensory parameter	A	B	C	D	E	F
Taste	6.20 ^b ±2.15	7.20 ^{ab} ±1.69	8.50 ^a ±0.71	7.50 ^{ab} ±1.27	7.60 ^{ab} ±1.17	8.10 ^{ab} ±1.73
Colour	7.44 ^a ±2.19	7.00 ^a ±1.70	7.90 ^a ±1.19	7.20 ^a ±1.62	7.20 ^a ±1.32	7.10 ^a ±1.37
Flavour	5.80 ^a ±1.55	6.60 ^a ±1.51	7.60 ^a ±1.07	7.20 ^a ±1.23	7.40 ^a ±1.71	7.67 ^a ±1.58
Mouthfeel	7.10 ^a ±1.45	7.10 ^a ±1.66	7.90 ^a ±1.20	6.60 ^a ±1.90	7.00 ^a ±1.56	7.30 ^a ±1.64
Overall Acceptability	6.40 ^a ±2.41	6.20 ^a ±2.15	7.80 ^a ±1.23	6.90 ^a ±1.29	7.60 ^a ±1.43	7.70 ^a ±1.64

Values are means ± Standard Deviation of duplicate determinations. Means in the same rows with different superscript are significantly different at $p < 0.05$

Conclusion and Recommendation

Blending zobo drink with *Justicia carnea* extract have shown to have improved the nutrient content of the drink. The iron content and iron bio-accessibility of zobo produced with *Justicia carnea* blends also increased. The taste and flavour of the zobo drinks were also improved by the inclusion of *Justicia carnea*.

It is recommended to consume this beverage with high protein foods, as it can improve the cumulative protein intake. More research on including *Justicia carnea* extract in our day-to-day meal is also encouraged and recommended.

Declaration of Competing Interest

The authors declare no conflict of interest.

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