Phytochemical and Mineral Properties of Zobo Drink Processed with Miracle Leaf and Wonderful Kola Extract

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ABSTRACT

This study investigated the phytochemical and mineral compositions of Zobo drink fortified with varying concentrations of miracle leaf and wonderful kola extracts. A mixture design was used generating five samples. Phytochemicals and mineral analysis were carried out on the samples using standard methods. Phytochemical analysis revealed significant variations with saponin content ranging from 0.20 to 1.20 mg/g, alkaloid from 0.07 to 1.00 mg/g, flavonoid from 14.10 to 19.59 mgQE/g, phenol from 16.02 to 26.25 mgGAE/g and tannin from 20.95 to 31.30 mg/g. Sample Bsc (60 % Zobo, 8 % miracle leaf extract, and 32 % wonderful kola extract) showed the highest levels of saponin (1.20 mg/g), alkaloid (1.00 mg/g), and tannin (31.30 mg/g), indicating its potential for cholesterol-lowering, immune-boosting, and antioxidant properties. Mineral analysis indicated significant increases in calcium (12.85–62.01 mg/100g), magnesium (38.83–84.79 mg/100g), iron (2.12–3.61 mg/100g), and sodium (10.20–17.45 mg/100g). Sample Bsc- (60% Zobo, 8% miracle leaf extract, and 32% wonderful kola extract), consistently showed the highest concentrations of these minerals, making the fortified drink particularly beneficial for bone health, iron supplementation, and cardiovascular function fortifying Zobo drink with miracle leaf and wonderful kola significantly enhances its nutritional and medicinal properties, offering a functional beverage with improved antioxidant activity, enhanced mineral bioavailability and therapeutic potential.

INTRODUCTION

Zobo drink is a non-alcoholic drink made from petals of *Hibiscus sabdariffa* flower (Alaribe *et al.*, 2018). In Nigeria, it is mainly cultivated in the northern part of the country, which includes Kano, Maiduguri, Jos, Katsina, and Kwara states (Nwodo *et al.*, 2008). The calyces of *Hibiscus sabdariffais* are a considerable source of nutrients such as carbohydrates, fibre, vitamin C, calcium and iron (Alaribe *et al.*, 2018). Zobo also contains antioxidants such as beta-carotene, vitamin C (Otutu *et al.*, 2012) and phytochemicals such as flavonoids (Umar *et al.*, 2014). Zobo is usually prepared by extracting the content of the calyces of *Hibiscus sabdariffa* with hot boiling water (Saidu *et al*., 2016). The shelf life of the Zobo drink is estimated to be between 24- 28 hours if it is not refrigerated (Ogunlana and Ogunlana, 2011).

Wonderful kola (*Buchholzia coriacea*) has numerous medicinal values. The seed gave it its common name (wonderful kola) because of its usage in traditional medicine. The parts of the plant usually eaten are the seeds, which can either be eaten raw or cooked (Nwachukwu *et al.*, 20l4). In Africa, wonderful kola has the ability to stop migraine headaches when applied on the forehead Ogunlana and Ogunlana (2011). The seeds, which have a peppery taste are used as a substitute for capsicum pepper. The seed is chewed as a substitute for kola nut (Nwachukwu *et al.*, 20l4).

Miracle leaf (*Bryophyllum pinnatum Lam*) belongs to the family Crassulaceae and is commonly known as Canterbury-bells, love plant, miracle leaf, and life plant (Umar *et al.,* 2014). The leaves and bark are not sweet, astringent to the bowels, analgesic, and helpful in diarrhoea and vomiting (Otutu *et al.*, 2012). Studies have shown the role of these phytochemicals (Saponin, tannin, alkaloids) in some medicinal plants on the central nervous system activities (Alaribe *et al.,* 2018). The leaf extract of miracle leaves showed some significant effects, which indicate substantial antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* at different levels (Nwachukwu *et al.,* 20l4).

MATERIALS AND METHODS

Sources of Raw Material

The Zobo leaf, wonderful kola and other materials was purchased from Eke-Awka market, Awka South Local Government. Anambra State. The Miracle leaf was obtained from Nnamdi Azikiwe University Awka. All the leaves were identified at the Botany Department, Nnamdi Azikiwe University Awka, Nigeria. The reagents and equipment were obtained from the Laboratory of the Departments of Food Science and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Experimental Design

Mixture design was used with design-expert version 6.0.10 (Stat Ease Minneapolis, Minn). The mixture components, A (Zobo calyxes), B (Miracle leave), and C (Wonderful kola extract) were summed up to 100. A total of six runs was generated, with the last sample serving as the control, as shown in Table 1

Table 1: Experimental design for Zobo calyxes, Miracle Leaf and Wonderful Kola extract

Samples	Zobo calyxes (ml)	Miracle leaf extract (ml)	Wonderful kola extract (ml)
Asc	70.00	10.00	20.00
Bsc	60.00	8.00	32.00
Csc	80.00	6.00	14.00
Dsc	76.00	18.00	6.00
Control	100	0.00	0.00

Sample preparation

Preparation of miracle leaf extract

The miracle leaf was processed into an extract, according to Nwodo *et al.* (2008). It was washed, and reduced to smaller sizes and then boiled in a hot water bath at 60^{o}C for 30 minutes. The liquid was strained through a fine mesh sieve 2 mm remove solid particles and stored at 4^{o} C for further studies.

Preparation of wonderful kola extract

According to Alaribe *et al.,* (2018), the wonderful kola seeds were processed into an extract. They were washed, dehulled, reduced to smaller sizes (0.5 g), dried using a dehydrator at 50^{0}C and milled into fine flour using an electric blender. Distilled water was then added to the wonderful kola flour and boiled in a hot water bath at 60^{0} C for 1 hour. The extract was then filtered using Whatman filter paper and stored at 4^{o}C for further studies.

Zobo processing procedures

The zobo drink was processed according to Ijarotimi (2015). The dried zobo calyxes were inspected and sorted to remove sticks, stones and other foreign material. The dried zobo calyxes were then rinsed under running water. The weight of the zobo calyxes were measured (Table 1) and added to 1 litre of boiling water along with the weight of the ginger, cloves and pineapple peel. It was cooked for about 20 minutes. The filtrate was obtained by filtration with a clean muslin cloth, and the residue was discarded. The wonderful kola and miracle leaf extracts were then added to each of the samples as stated in the experimental design, and the samples were then hot-filled and packaged in sterilized bottles. The sample was cooled at room temperature and refrigerated at 28^{o}C

Method of Analysis

Qualitative analysis of phytochemicals The Flavonoid, Saponins, Alkaloids, Phenol and Tannin Content were determined using the standard methods described by the AOAC (2010).

Mineral Analysis
Magnesium, Calcium, Iron and Sodium content were determined using the standard methods described by the AOAC (2010).

Statistical Analysis

Data generated from the respective analysis was compiled appropriately and subjected to analysis of variance (ANOVA). The mean of the data was separated using Duncan Multiple Range Test (Statistical Product and Service Solution package version 30).

RESULTS AND DISCUSSION

The results of the phytochemical analysis of Zobo drink processed with different concentrations of miracle leaf and wonderful kola extracts are presented in Table 2; the saponin content ranged from 0.20 to 1.20 mg/g, Alkaloid ranged from 0.07 to 1.00 mg/g, flavonoid ranged from 14.10 to 18.15 mgQE/g, phenol ranged from 16.02 to 20.98 mgGAE/g, Tannin ranged from 22.16 to 31.30 mg/g. Each of these compounds is linked to significant health benefits and plays an essential role in the medicinal properties of plant-based beverages (Otutu *et al.*, 2012).

Saponin content varied significantly across the sample, with sample Bsc-(60% Zobo, 8 % miracle leaf extract and 32 % wonderful kola extract) recording the highest concentration (1.20mg/g). In contrast, the control, which contains 100 %) Zobo, showed the lowest (0.20 mg/g). There were significant differences ($p \leq 0.05$) in all the samples analyzed. The findings of this work suggest that both miracle leaf and wonderful kola are key contributors to saponin levels. Saponins are known for their cholesterol-lowering and immune-boosting properties (Otutu *et al.*, 2012). Adeniji (2017) reported a saponin content of 0.15 mg/g for the zobo drink, which is actually lesser than the result obtained in this study.

Alkaloid content ranged from 0.07 to 1.00 mg/g, with sample Bsc-(60% Zobo, 8% miracle leaf extract and 32% wonderful kola extract) having the highest value while the control had the lowest value. There were significant differences ($p \leq 0.05$) in all the samples analysed. The notable increase in alkaloids in the supplemented samples could indicate a potential boost in these medicinal benefits when Zobo is mixed with miracle leaf and wonderful kola; this study is in agreement with the findings of Otutu *et al.*, (2012), which reported a higher alkaloid content for wonderful kola.

Flavonoid content, which is linked to antioxidant activity, was highest in the control (19.59 mgQE) and lowest (14.10 mgQE/g) in sample Bsc- (Zobo-60, miracle leave extra- 8, Wonderful kola- 32). There were significant differences ($p \leq 0.05$) in all the samples analyzed. Surprisingly, the supplemented sample had a significantly lower flavonoid content despite its high saponin and alkaloid levels. This suggests a trade-off between the addition of miracle leaf and wonderful kola and the concentration of flavonoids in Zobo. Flavonoids are known to reduce the risk of chronic diseases such as heart disease (Ogunlade *et al.*, 2021). Ogunlade *et al.* (2021) reported a higher flavonoid content of 20.45 to 22.60 mgQE/g. The variation in the flavonoid content could be due to the differences in the raw materials used.

Phenol content, which is associated with anti-inflammatory and antioxidant properties, was highest in the control (26.25 mg/g), with a decreasing trend across other samples. This could suggest that the addition of miracle leaf and wonderful kola slightly reduces Zobo's phenol content. However, sample Csc- (Zobo- 80, miracle leave extra- 06, Wonderful kola- 14) still maintained a relatively high (23.43 mg/g) phenol concentration, indicating that a moderate combination of these extracts could balance the phenol content. Omemu *et al*. (2006) reported a range of 23.50 to 25.99 mg/g for Zobo drinks, which is in agreement with the findings of this study.

Tannins, known for their antimicrobial and anticancer potential, were found in the highest (31.30 mg/g) concentrations in the sample Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32), significantly higher than the control (20.95 mg/g). This suggests that the addition of wonderful kola particularly boosts tannin

content, which could enhance the health benefits of the Zobo drink. This study is in agreement with the findings of Otutu *et al.,* (2012), which reported a higher tanning content for wonderful kola.

Table 2: Phytochemical properties of Zobo drink processed with miracle leaves and wonderful kola extract.

Sample	Saponin (mg/g)	Alkaloid (mg/g)	Flavonoid (mgOE/g)	Phenol (mgGAE/g)	Tanning (mg/g)
Abs	0.74^{b}±0.02	0.83^{b}±0.03	15.40^{d}±0.45	20.98^{c}±0.08	27.98^{b}±0.10
Bsc	1.20^{a}±0.02	1.00^{a}±0.02	14.10^{e}±0.14	16.02^{e}±0.03	31.30^{a}±0.09
Csc	0.38^{d}±0.03	0.19^{d}±0.01	18.15^{b}±0.05	23.43^{b}±0.41	22.16^{d}±0.02
Dsc	0.58^{c}±0.02	0.41^{c}±0.04	16.09^{c}±0.04	18.34^{d}±0.03	25.00^{c}±0.08
Control	0.20^{e}±0.02	0.07^{e}±0.01	19.59^{a}±0.26	26.25^{a}±0.24	20.95^{e}±0.07

Values are mean ± standard deviation of triplicate determinations. Means in the same column with different superscripts are significantly different (p≤0.05). Key: Abs- (Zobo- 70, miracle leave extra- 10, Wonderful kola- 20) Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32), Csc - Zobo- 80, miracle leave extra- 06, Wonderful kola- 14), Dsc (Zobo- 76, miracle leave extra- 18, Wonderful kola- 06), Control Zobo- (100 miracle leave extra).

The results of the mineral content of Zobo drink fortified with miracle leaf and wonderful kola extracts is presented in Table 3. The addition of these extracts significantly influenced the mineral composition of the drink across the different samples. The calcium content ranged from 12.85 to 62.01 mg/100g, magnesium ranged from 38.83 to 84.79, iron ranged from 2.12 to 3.61mg/100g, and Sodium ranged from 10.20 to 17.45 mg/100g.

Calcium is essential for bone health, muscle function, and cardiovascular health (Mohd-Esa and Abdul-Rahman, 2010). The results of the calcium content show that sample Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32) had the highest value (62.01mg/100g) while the control sample had the lowest (12.85 mg/100g). There were significant differences (p≤0.05) in all the samples analyzed. The increase in calcium content in supplemented samples can be attributed to the high amount of wonderful kola extract, which is known to be rich in minerals. The higher calcium levels could make the product particularly beneficial for individuals seeking to improve bone health and reduce the risk of osteoporosis (Mohd-Esa and Abdul-Rahman, 2010). The results of this work are higher than the values (10 to 36 mg/100g) reported by Mohd-Esa and Abdul-Rahman (2010) for calcium content Roselle (*Hibiscus sabdariffa L*.) seed.

Magnesium plays a key role in over 300 enzymatic reactions in the body, including those related to muscle and nerve function, blood sugar control, and blood pressure regulation (Osueke and Ehirim, 2014). Sample Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32) had the highest magnesium content (84.79 mg/100g), significantly higher than the control (38.83 mg/100g). The values of this research are higher than 32.65 to 43.11mg/100g, as reported by Olayemi *et al*. (2011). The high magnesium content in the fortified Zobo samples could offer benefits related to cardiovascular health and the prevention of metabolic disorders like diabetes (Osueke and Ehirim, 2014).

Iron is crucial for oxygen transport in the blood and energy metabolism. (Samy, 2018). Sample Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32) had the highest value (3.61 mg/100g), while the control sample recorded the lowest iron content (2.12 mg/100g).

The fortification of Zobo with wonderful kola and miracle leaf boosts its iron content, enhancing its potential as a dietary supplement for iron, especially for women and individuals with iron-deficiency anaemia. The values of this work are higher than the values 1.25 to 2.10 mg/100g reported by Ijarotimi *et al.* (2015) for commercial ready-to-drink bottled Zobo drink products; the variation in the results might be due to differences in raw materials used.

Sodium is important for fluid balance and nerve function, but excessive intake can lead to hypertension (Samy, 2018). Sample Bsc – (Zobo-60, miracle leave extra- 8, Wonderful kola- 32) had the highest value (17.45 mg/100g) while the control had the lowest value (10.20 mg/100g). While sodium is necessary in small amounts, the moderate levels seen in the Zobo drink variants. The results of this work are in agreement with the values (11.16 to 15.20 mg/100g) reported by Kehinde and Augustine (2022) for the sodium content of 'Zobo' Drink fortified with Pineapple and Watermelon.

Table 3: Mineral composition of Zobo drinks processed with miracle leaves and wonderful kola extract.

Sample	Calcium (mg/100g)	Magnesium (mg/100g)	Iron (mg/100g)	Sodium (mg/100g)
Asc	46.88^{b}±0.01	68.06^{b}±0.01	3.14^{b}±0.02	15.22^{b}±0.01
Bsc	62.01^{a}±0.01	84.79^{a}±0.03	3.61^{a}±0.01	17.45^{a}±0.01
Csc	35.39^{c}±0.03	59.12^{c}±0.01	2.84^{c}±0.01	13.62^{c}±0.00
Dsc	33.06^{d}±0.01	49.17^{d}±0.01	2.71^{d}±0.02	13.18^{d}±0.01
Control	12.85^{e}±0.01	38.83^{e}±0.02	2.12^{e}±0.01	10.20^{e}±0.01

Values are mean ± standard deviation of triplicate determinations. Means in the same column with different superscripts are significantly different (p≤0.05). Key: Asc- (Zobo- 70, miracle leave extra- 10, Wonderful kola- 20) Bsc - (Zobo-60, miracle leave extra- 8, Wonderful kola- 32), Csc - Zobo- 80, miracle leave extra- 06, Wonderful kola- 14), Dsc (Zobo - 76, miracle leave extra- 18, Wonderful kola- 06), Control Zobo- (100, miracle leave extra).

CONCLUSION

This study explored the impact of incorporating miracle leaf and wonderful kola extracts on the phytochemical and mineral composition of the Zobo drink. Results showed that the inclusion of these extracts significantly enhanced the levels of beneficial compounds like saponins, alkaloids, and tannins, which are known for their health-promoting properties. Although the flavonoid and phenol contents were reduced in supplemented samples, their concentrations remained within ranges beneficial for health and contributing to enhanced antioxidant and immune-boosting potential. Mineral analysis revealed significant increases in calcium, magnesium, and iron, which are essential for bone, cardiovascular, and metabolic health. Overall, fortifying Zobo with miracle leaf and wonderful kola offers potential as a functional beverage with added nutritional and health benefits.

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