

Anti-diabetic potentials of soursop, morinda lucida and miracle leaves extracts fortified with tigernut beverages

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Abstract

Diabetes is primarily managed through treatment rather than prevention. Exploring natural therapies such as medicinal plant leaves extract with anti-diabetic properties, may provide a complementary approach. This study evaluated the antioxidant, anti-diabetic, nutritional, physicochemical, and sensory properties of tigernut beverages fortified with leaf extracts (soursop, *Morinda lucida* and miracle leaf). The leaf extract was obtained via the decoction method. Beverages were formulated using 85 ml of Tigernut milk and 5 ml of each leaf extract with 100% Tigernut as control. Samples were analyzed following AOAC methods, while α -amylase and α -glucosidase inhibition assays assessed anti-diabetic potential. Sensory evaluation was conducted with 50 trained panellists. The results showed that tigernut beverages exhibited significantly higher proximate values than soursop, *Morinda lucida*, and miracle leaves extract, while all extracts were richer in vitamins A, B₁, B₃, and C. Mineral content increased in fortified beverages, and tigernut milk (TGN) appeared lighter in colour. The fortified beverages extracts were low in acidic with pH (5.50–6.46). Phenolic and flavonoid contents ranged from 0.40–1.08 mg/100 ml and 0.16–0.96 mg/100 ml, respectively, influencing the phenolic profile of the beverages (0.13–0.62 mg/100 ml). All samples significantly enhanced DPPH and FRAP scavenging activities. Fortified beverages showed strong inhibitory effects on α -amylase (10.81–60.41%) and α -glucosidase (14.65–17.56%), indicating potential anti-diabetic properties. Sensory evaluation revealed that tigernut beverages with soursop extract (TGNSSE) had the highest consumer acceptance and offered the best nutritional and health benefits.

1.0 Introduction

Diabetes is a chronic metabolic disorder that is currently managed primarily through treatment rather than prevention. With the rising prevalence of diabetes, exploring natural therapies, such as medicinal plant extracts with anti-diabetic properties, may provide a complementary approach to conventional treatment. Medicinal plants have been widely used in traditional medicine to manage various ailments due to their bioactive compounds, affordability, and relative safety compared to synthetic drugs [1, 2]. The World Health Organization (WHO) recognizes the therapeutic potential of herbal remedies, and researchers continue to investigate their medicinal properties [3]. Functional beverages are non-alcoholic drinks formulated to provide health benefits beyond basic nutrition. These beverages are often enriched with bioactive compounds such as vitamins, minerals, antioxidants, probiotics, and plant extracts, offering potential health benefits such as improved digestion, enhanced immune function, and chronic disease management [4]. The growing consumer demand for healthier dietary choices has led to an increased interest in functional beverages, which are now widely available in various formulations, including dairy-based drinks, fruit juices, herbal infusions, and plant-based milk alternatives [5]. Tigernut (*Cyperus esculentus*) has gained attention due to its high nutritional value and health-promoting properties. Tigernut milk is rich in dietary fiber, essential minerals, and natural antioxidants, making it an excellent alternative to dairy-based beverage [6]. When combined with medicinal plant extracts, tigernut-based beverages may provide enhanced functional benefits, including improved glycemic control,

cardiovascular health, and gut microbiota modulation [7]. *Bryophyllum pinnatum* (family: *Crassulaceae*), as shown in Fig. 1a, is also known as *Kalanchoe pinnatum* or *Bryophyllum calycinum*, is extensively used in traditional medicine [8]. It has a sour taste, hot strength and sugary post digestive effect. The herb contains a wide range of valuable chemicals that could be responsible for its various pharmacological effects [9]. Anti-leishmanial, anticancer, immunological suppressive, antiulcer, anti-inflammatory, anthelmintic, antihistaminic, antifungal, analgesic, antihypertensive, anti-diabetic, and anti-mutagenic activities have all been demonstrated for the leaves of the *Bryophyllum pinnatum* plant. The herb was used to alleviate menstrual issues and uterine contractions in India [10].

Annona muricata L. as shown in Fig. 1b, is the scientific name for it, and it is a member of the *Annonaceae* family [12, 13]. The soursop fruit is known as "evo" in Ewe, "aborofontungu" in Twi, and "aluguntugui" in Ga in Ghana. Due to potential health benefits, soursop is grown in much of the world [12]. According to [13], the fruit has an ovoid, dark green, spiky shape with a juicy, acidic, white, and aromatic pulp. Soursop is gaining global popularity due to its medicinal, nutritional, flavour, and colour properties [14]. *Annona muricata* L. has been associated to a variety of health advantages, including antioxidant properties [15].

Morinda lucida is a medium-sized tree that grows to a height of around 15 meters and is frequently utilized in traditional West and Central African medicine. In traditional medicine, *Morinda lucida* as shown in Fig. 1c, is used to treat fevers, jaundice, hypertension, cerebral congestion, diarrhea, diabetes, and gastric ulcers [16, 17]. reported that the leaves and roots of *Morinda lucida* have hypoglycaemic anti-diabetic properties. *Morinda lucida* extracts have been shown to have activities against *Salmonella typhi* [18] isolated uterine smooth muscle contractility [19], toxicity and mutagenic studies [20, 21] and reducing and antioxidant properties [22]. It contains several secondary metabolites that give the plant its ethnomedical properties, including alkaloids, tannins, saponin, flavonoids, and phenols, as well as two potent antioxidants, vitamins A and E, which may be helpful in the treatment of degenerative diseases like atherosclerosis. Studies have demonstrated the anticancer, hypertensive, cytotoxic, genotoxic, anti-spermatogenic, hypoglycemic, and anti-diabetic effects of the leaf and stem bark. The leaves effectively treat and ameliorate infertility in women of all kinds. *Morinda lucida* is applied topically to treat wound infections, cancer, insomnia, jaundice, and irregular menstruation [23] .

With the enormous health benefit of miracle leaf, *Morinda lucida* and sour sop plant, it has limited usage in formulation and beverages. To further enhanced the value addition and usage of the medicinal plant, this study explores the development of functional beverages enriched with medicinal plant extracts, focusing on their antioxidant, anti-diabetic, and nutritional properties. By assessing the physicochemical, proximate, vitamin, and mineral composition of these beverages, as well as their sensory attributes, this research aims to determine their potential as a natural and effective dietary intervention for managing diabetes and promoting overall health.

2.0 Materials and methods

2.1. Sources of raw materials

The medicinal plants used in this study (fresh soursop, miracle leaf, and *Morinda lucida*) were obtained from the streets of Bioku, Iyana-Agbala, Adeggbayi, Ibadan, Oyo State, Nigeria (Latitude: 7.3644°N, Longitude: 4.0009°E). Tigernuts were purchased from Gbagi Market, Ibadan, Oyo State, Nigeria (Latitude: 7.3927°N, Longitude: 3.9550°E). All chemicals used in the study were of analytical grade.

2.2.1. Extraction of soursop, miracle leaf and *morinda lucida* leaves

The decoction method was used to extract soursop, miracle leaf, and *Morinda lucida* leaves, following a modified procedure of [24]. As shown in Fig. 2a, approximately 1 kg of fresh leaves from each plant leaves were rinsed thoroughly with clean water to remove dirt and dust. The leaves were then boiled in 500 ml of distilled water at 80°C for 15 min before being allowed to cool. The extract was separated using a sterile muslin cloth and further filtered through sterile Whatman filter paper.

2.2.2. Production of tigernut milk

The tigernut milk was prepared using a modified method of [7], as shown in Fig. 2b. About 1kg of tigernut were washed twice in water, drained, and then blended into a paste with 250 ml of distilled water using a laboratory blender. The resulting paste was filtered through a muslin cloth with a 1 mm mesh size to obtain the tigernut extract.

2.2.3. Formulation of Tigernut Beverages with Medicinal Plant leaves Extract

The tigernut beverage (tigernut milk with extract soursop, miracle leaf, and *Morinda lucida* leaves) blends were prepared according to the method of [7]. The samples were pasteurized at 70°C for 10 min, then cooled and used for further analysis. The beverages were freshly prepared for the analysis as presented in Fig. 2c.

2.3. Determination of Proximate Composition

Moisture, protein, fat, crude fiber, and ash contents were determined using the procedures described by [25]. Carbohydrate content was calculated by difference (%).

2.4. Determination of Vitamin A, C and B Complex

The method of [23] was used to determined vitamin A, High-Performance Liquid Chromatography (HPLC) was used which involves sample extraction with an organic solvent, followed by saponification to remove lipids. The extract was then filtered and injected into an HPLC system equipped with a UV or fluorescence detector. A C18 reverse-phase column was used, with a mobile phase consisting of methanol, acetonitrile, and water. Quantification was achieved by comparing peak areas with those of known vitamin A standards.

The determination of vitamin C and B-complex in beverage samples was effectively carried out using High-Performance Liquid Chromatography (HPLC) with a C18 reversed-phase column and UV detection at specific wavelengths. Sample preparation involves acid extraction followed by filtration, with vitamin C stabilized using metaphosphoric acid and B-complex vitamins extracted using hydrochloric acid. The mobile phase typically consists of phosphate buffer and methanol, with detection wavelengths ranging from 246 nm to 361 nm, depending on the vitamin. Quantification was achieved through calibration curves using standard solutions [23].

2.5. Determination of Mineral Analysis

The mineral elements (Ca, Na, P, and Fe) in the extracts and tigernut beverages were determined using the method described by [27]. For digestion, 10 ml of nitric acid and HCl were mixed with 0.5 g of the sample in a fume hood for 10 min. The digested mixture was then filtered, and the filtrate was diluted to 50 ml with distilled water. An Atomic Absorption Spectrophotometer (Perkin Elmer, Model 402, England) was used to assess calcium, magnesium, and iron concentrations, while sodium and potassium levels were measured using flame photometry. Phosphorus was determined using a UV spectrophotometer, following the Vanado-molybdate technique. A 2:1 mixture of 2 ml nitric acid and phosphate solution was treated with vanado-molybdate reagent. The mixture was left to stand for 10 minutes before the absorption was measured at 417 nm.

2.6. Determination of Colour Analysis

The colour analysis of the extracts and tigernut beverage was conducted as previously described by [28]. A colorimeter PCE-CSM (Deutschland GmbH) was calibrated against a white plate and used with CQCS3 software (Shenzhen 3nh Technology, China) to take colour readings. The aperture of the spectrophotometer was placed on top of the sample and readings were taken and expressed on the L*, a*, b* tristimulus scale, ΔE was calculated using mathematical formula:

$$\Delta E = \sqrt{((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)}$$

Where ΔE = Colour difference (Delta E), ΔL^* =Lightness difference, Δa^* = Red-green axis difference, Δb^* =Yellow-blue axis difference.

2.7. Determination of pH

The pH of the extracts and tigernut beverages was measured using the Mettler Toledo MP220 pH meter (Greifensee, Switzerland). The volumetric titration method was employed to measure the titratable acidity.

2.8. Total Phenolic and Flavonoids Contents

The Folin-Ciocalteu method described by [29] was used to determine total phenolic content of the extracts and beverages. Tannic acid was used as standard and the result expressed as in percentage.

The total flavonoid content of juice extracts and tigernut beverages was measured using the [30] method. Quercetin was used as a standard and the flavonoid content was expressed as mg/100 g.

2.9. Determination of Free Radical Scavenging Ability (DPPH).

The method of [31] was used to determine the DPPH radical scavenging assay to assess the scavenging capability of the beverages. 1 ml of 0.04 mM methanolic solution of DPPH was mixed with equal volume of the beverage and the mixture was incubated for 30 min in the dark. The absorbance of the mixture was measured at 516 nm in a UV-visible Spectrophotometer. The DPPH reagent without sample extract was used as a control. The extracts was calculated as % inhibition as follows:

Percentage inhibition

Where A_c = Absorbance of control. A_e = Absorbance of extract.

2.10. Determination of Ferric Reducing Antioxidant Power (FRAP)

The method of [32] was used to test the ferric-reducing antioxidant potential of extracts and tigernut beverages. The FRAP reagent was made by combining acetate buffer, 2, 4, 6-Tris-(2-pyridyl)-s-triazine (TPTZ), and $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. Dilute 0.3 ml of juice extract with 0.7 ml of distilled water, then add 2.85 ml of FRAP reagent. After 20 min of reaction at $50^\circ C$, the absorbance was measured at 700 nm. To estimate the antioxidant capacity of juice extract samples, a standard curve was prepared using ascorbic acid.

2.10. Determination of α -amylase inhibition

The extracts and beverages were tested for inhibitory activity on α -amylase using [33] spectrophotometric technique, with slight modifications.

100 μ L of diluted material was mixed with an equal volume of 20 mM sodium phosphate buffer (pH 6.9 and 6 mM NaCl) containing 1 mg/ml porcine pancreatic α -amylase (EC 3.2.1.1). After 10 min of incubation at $25^\circ C$, the mixture was treated with 200 μ L of dinitrosalicylic acid reagent. To stop the reaction, it was incubated in a boiling water bath for 5 min, then cooled in cold water and diluted further. The absorbance of the samples was determined using a spectrophotometer at 540 nm. The control sample contained all of the reagents but did not include the test sample. The α -amylase inhibitory activity was calculated as follows:

Inhibition of α -amylase activity (%) =

2.11. Determination of α -glucosidase inhibition

The leaf extracts and tigernut beverages were tested for α -glucosidase inhibitory activity using the method described by [34]. A substrate solution of 3 mM p-nitrophenyl α -D-glucopyranoside (pNPG) was

prepared in 100 mM phosphate buffer (pH 6.9), and α -glucosidase from *Saccharomyces cerevisiae* (0.5 mg/ml) was also dissolved in the same buffer (pH 6.8). 50 μ L of the sample was diluted and 50 μ L of α -glucosidase was added. The activity of α -glucosidase was measured spectrophotometrically at 405 nm using the yellow-coloured p-nitrophenol generated from NPG. The control sample contained the full reagent except for the test sample. The inhibitory activity was then estimated as follows:

Glucosidase inhibition (%) = $\frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$

2.12. Sensory Evaluation of Tigernut Beverages

The method of [35] was used for performing sensory evaluations. 50 panelists consisting adults, youth, male and female were seated in well-illuminated booths and given with coded samples. Panelists used water to rinse their palate after each test. The beverage's colour, taste, flavour, appearance, and overall acceptability were evaluated using a nine-point Hedonic scale. The following numerical values were utilized to score dislike extremely = 1, very much = 2, dislike moderately = 3, dislike slightly = 4, neither like nor dislike = 5, like slightly = 6, moderately = 7, like very much = 8, and like extremely = 9.

2.13. Statistical Analysis

Data analysis was performed using SPSS version 20.0 All the analyses were carried out in triplicates and data was analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test. Statistically significant data have $p < 0.05$.

3.0 RESULTS AND DISCUSSION

3.1 Proximate Composition of the Extracts and Beverages

Table 1 present the proximate composition of the leaf extracts and tigernut beverages fortified with extracts. The moisture content of the extracts ranged from 93.33 to 99.54%, while the tigernut beverages ranged from 89.10 to 93.66%. The results indicate that the moisture content of the extract samples was higher than that of the tigernut beverages. The moisture content found in this study is higher than the 72.26% reported for soursop extract by [36]. Among the tigernut beverages, the control sample (100% tigernut TGN) had the lowest moisture content (89.10%), while tigernut with soursop extract had the highest (93.66%). This suggests that the addition of extracts increased the moisture content of the tigernut beverages compared to the control sample.

The crude protein content of the extracts ranged from 0.06 to 0.22%, while the tigernut beverages contained between 1.22 and 2.36%. Among the extracts, *Morinda lucida* (MME) had the highest protein content (0.22%). The control sample exhibited the highest protein level (2.36%) among all samples. Among the fortified tigernut beverages, tigernut with *Morinda lucida* had the highest protein content (1.61%). Significant differences ($p < 0.05$) were observed among the samples. The high protein concentration in tigernut milk with *Morinda lucida* may be attributed to the high amino acid content of both tigernut milk and *Morinda lucida* leaves.

The crude fat content of the tigernut beverages ranged from 0.42 to 1.62%. The control sample had the highest crude fat content (1.62%), whereas tigernut with *Morinda lucida* had the lowest (0.42%). The crude fat content of the control sample could be attributed to the high unsaturated fatty acid content of tigernut milk, which is similar to that of olive oil, as reported by [37]. Due to its high moisture and fat content, refrigeration is necessary to prevent spoilage. The fiber content of the tigernut beverages ranged from 0.08 to 0.26%. Among the beverages, tigernut milk with miracle leaf extract had the highest fiber content (0.26%), while tigernut with *Morinda lucida* had the lowest (0.08%). These results suggest that the addition of extracts to tigernut beverages reduced fiber content.

The ash content of the tigernut beverages ranged from 0.11 to 0.33%. Among the tigernut beverages, tigernut with soursop, miracle leaf, and *Morinda lucida* had the highest ash content (0.33%). The carbohydrate content of the extracts ranged from 0.40 to 6.53%, while the tigernut beverages contained between 4.07% and 6.53%. Among the extracts, *Morinda lucida* had the highest carbohydrate content. Among the tigernut beverages, tigernut with miracle leaf had the highest carbohydrate content. The carbohydrate levels in this study were higher than those reported by [38], who found carbohydrate levels ranging from 2.07 to 4.33% in tigernut milk supplemented with *Moringa oleifera* extracts. Tigernut milk has been traditionally used to treat gas, indigestion, diarrhea, and dysentery due to its high carbohydrate content [6].

3.2. Vitamin Composition of the Extracts and Beverages

Table 2 show the vitamin composition of the leaf extracts and tigernut beverages. Vitamin A levels in the extracts ranged from 0.52 to 2.12 mg/100 g, while in the tigernut beverages, varied between 0.03 and 0.30 mg/100 g. Among the extracts, soursop extract had the highest vitamin A content (2.12 mg/100 g). The addition of leaf extracts to tigernut beverages influenced their vitamin A levels. [39] reported a vitamin A content of 0.48 mg/100 g in miracle leaf, which is lower than the value found in this study for miracle leaf extract (0.52 mg/100 g). The high vitamin A content observed in both the extracts and tigernut beverages highlights its essential role in maintaining vision, supporting immune function, and ensuring proper heart and lung function.

Vitamin B₁ levels in the leaf extracts ranged from 5.67 to 8.60 mg/100 g, while in the tigernut beverages, they varied from 0.02 to 1.21 mg/100 g. Among the extracts, *Morinda lucida* extract had the highest vitamin B₁ concentration (8.60 mg/100 g). This also impacted the tigernut beverage fortified with *Morinda lucida*, which had the highest vitamin B₁ content (1.21 mg/100 g) among all tigernut beverage samples. Vitamin B₁, a water-soluble vitamin, is essential for converting carbohydrates into glucose for energy production [39]. Vitamin B₃ levels in the extracts ranged from 0.25 to 0.26 mg/100 g, while in the tigernut beverages, they varied between 0.02 and 0.06 mg/100 g. Among the extracts, soursop extract had the highest vitamin B₃ content. The tigernut beverages contained only trace amounts of vitamin B₃.

Vitamin C levels in the extracts ranged from 0.07 to 0.12 mg/100 g, whereas in the tigernut beverages, they ranged from 0.01 to 0.13 mg/100 g. Among the extracts, *Morinda lucida* had the highest vitamin C

content (0.12 mg/100 g), which may contribute to enhanced immune function. Among the tigernut beverages, the tigernut milk fortified with miracle leaf had the highest vitamin C content (0.10 mg/100 g), whereas the tigernut beverage fortified with soursop extract had the lowest vitamin C content. Significant differences were observed between the samples. [40] reported vitamin A, B₁, and C levels in soursop leaf extract to be 3.23, 0.23, and 2.08 mg/100 g, respectively, indicating inconsistencies with the results obtained in this study. [39] also highlighted the role of vitamin C in preventing various debilitating diseases and strengthening immune function.

3.3. Mineral Composition of the Extracts and Beverages

Table 3 show the mineral composition of the leaf extracts and tigernut beverages. The calcium levels in the extracts ranged from 635.50 to 2499 mg/L, while the tigernut beverages ranged from 621.74 to 1138.76 mg/L. The results indicate that miracle leaf extract had the highest calcium content (2499.30 mg/L) among the extract samples. Among the tigernut beverages, tigernut fortified with *Morinda lucida* extract had the highest calcium content (1138.76 mg/L). Calcium intake is essential, as its impairment in diabetes may contribute to cellular dysfunction, affecting cardiac muscles, erythrocytes, platelets, and skeletal muscles [41]. This impairment may also negatively impact insulin secretion and action, potentially leading to vascular complications [41]. However, the administration of these extracts may play a role in modulating immune responses and supporting insulin secretion in pancreatic beta cells. Calcium is also required for regulating vital organs, including the liver and heart. The calcium content in this study was higher than the values reported by [42], which ranged from 83.83 mg/100 g to 122.40 mg/100 g. Calcium is crucial for various physiological processes, such as muscle function in the heart and skeletal system, nerve signal transmission, blood clotting, and enzyme and hormone regulation [43]. The calcium levels observed in this study were also higher than those reported by [44], where tigernut milk fortified with moringa extract contained 139.07–140.34 mg/100 mL of calcium.

The potassium content of the extracts ranged from 1167.34 to 1598.87 mg/L, while the tigernut beverages contained between 838.66 and 2407.77 mg/L. Among the extracts, *Morinda lucida* MME had the highest potassium content (1617 mg/L), while tigernut milk with miracle leaf TGNMLE had the highest potassium content (2276.20 mg/L) among the tigernut beverages. Tigernut is known to be rich in essential minerals, including potassium, phosphate, and calcium[6]. High potassium intake is essential for normal cell function, neurotransmission, muscle contraction, and maintaining acid-base balance [45, 46].

The sodium and iron levels in the extracts ranged from 972.5 to 1413.63 mg/L and 287.65 to 1258.78 mg/L, respectively. In the tigernut beverages, sodium levels ranged from 1621.38 to 3613.78 mg/L, while iron levels varied from 164.32 to 1940.89 mg/L. Among the extract samples, MME had the highest sodium content (1413.63 mg/L), while tigernut milk with *Morinda lucida*, miracle leave and sour sop extract had the highest sodium content (3613.78 mg/L) among the tigernut beverages. The results showed significant differences between the extract samples and the tigernut beverages fortified with extracts. The potassium-to-sodium (K: Na) ratios of both the extracts and tigernut beverages were higher than the recommended minimum of 5:1 for optimal health [47]. Excess sodium intake has been linked to

hypertension and high blood pressure; therefore, consuming low-sodium, high-potassium fruits and vegetables is beneficial for overall health [48].

Iron levels were highest in the Miracle leaf extract (1258.78 mg/L) compared to other extracts, while tigernut milk with miracle leaf extract had the highest iron content among the tigernut beverages. Iron is an essential component of hemoglobin, facilitating oxygen and carbon dioxide transport. The iron levels observed in this study were significantly higher than the values reported by [42] (0.026 mg/100 g to 0.0028 mg/100 g) and [43], who reported iron levels of 4.12 mg/L and 0.87 mg/L for soursop leaf extract and ginger leaf extract, respectively. Adequate iron intake is crucial for preventing anemia and related disorders. Iron deficiency is commonly observed in individuals with diabetes, particularly type 2 diabetes, and can be associated with other micronutrient deficiencies, potentially leading to severe health complications. Poorly controlled diabetes is linked to increased oxidative stress and the accumulation of oxidation end-products, which can elevate blood glucose levels and weaken antioxidant defense mechanisms [43]. However, sequestration of iron-binding and storage proteins may contribute to the body's antioxidant defense system.

3.4. Colour Properties of the Extracts and Beverages

Colour is one of the most important factors influencing consumer acceptance of any new or improved food product [14]. Table 4 presents the colour parameters of the leaf extracts and beverage blends. The L^* value represents the lightness of the extracts and tigernut beverages. The extracts ranged from 29.47 to 29.49, whereas the tigernut beverages ranged from 47.49 to 48.89. The results demonstrate that Tigernut milk was significantly ($p < 0.05$) lighter than all other samples containing vegetable extracts. Among the tigernut beverages enriched with extracts, Tigernut milk with miracle leaf extract (48.89) had the highest L^* value. A similar result was observed in tigernut beverages fortified with *Vernonia amygdalina* and *Momordica charantia*, where tigernut with honey exhibited the highest L^* value (72.81) [7]. Additionally, chocolate-flavoured milk beverages showed a significant reduction in L^* values with increasing chocolate concentrations [49].

The a^* value represents the transition from red to green, influenced by chlorophyll content. The a^* values of the extracts ranged from -0.12 to -1.07, whereas the tigernut beverages ranged from 0.27 to 0.40. The results indicate that the leaf extracts had negative a^* values, signifying a shift towards green due to the high chlorophyll content of the leaves used in the formulations. Among the tigernut beverages, tigernut milk with *Morinda lucida*, miracle leave and sour sop extract TGNMMS (0.40) recorded the highest a^* value. Significant differences were observed between the samples when compared to other tigernut beverages ($p < 0.05$).

The b^* value represents the transition from blue to yellow. The b^* values of the extracts and beverages varied, ranging from -1.31 to -1.33 for the extracts and 0.11 to 1.96 for the tigernut beverages. The extracts exhibited negative b^* values, indicating a bluish tone, while Tigernut milk with soursop extract had the highest b^* value (1.96). Similar findings have been reported in fruit juices and plant-based milk

beverages [50, 7]. The tigernut beverages fortified with extracts displayed a more pronounced bluish hue compared to other samples.

The total colour difference (ΔE) of the leaf extracts ranged from 29.51 to 29.52, while that of the tigernut beverages ranged from 47.52 to 48.65. The ΔE values indicated that the miracle leaf extract (MLE) had the highest colour difference among the leaf extracts, whereas the tigernut beverage containing soursop, miracle leaf, and *Morinda lucida* had the highest colour difference (48.65) among the tigernut beverages. There were significant differences between the samples ($p < 0.05$).

3.5. Physico-chemical Properties of the Extracts and Beverages

Table 5 indicate the physico-chemical properties of the extracts and tigernut beverages. The total solids of the extracts ranged from 0.46 to 6.67%, while those of the tigernut beverages ranged from 6.61 to 10.92%. The results indicate that miracle leaf extract (6.67%) had the highest total solid content, whereas SSE (soursop extract) had the lowest (0.46%). Among the tigernut beverage samples, the highest total solid content was recorded at 6.61%. There were significant differences ($P < 0.05$) between the samples.

The total acidity of the extracts ranged from 0.27 to 0.61%, while that of the tigernut beverages ranged from 0.84 to 1.28%. *Morinda lucida* extract (0.61%) had the highest total acidity among the extracts, whereas. Tigernut milk with *Morinda lucida* extract (1.28%) had the highest total acidity among the tigernut beverages. The total acidity observed in this study was higher than the values reported by [7], who found that baobab-fortified tigernut beverages had total acidity levels ranging from 0.19 to 0.57%.

The pH levels of the extracts and tigernut beverages varied from 5.50 to 6.46 and 5.85 to 6.35, respectively. The results indicate that all samples were acidic. The study also revealed that tigernut milk fortified with leaf extracts had slightly lower pH values than the control sample, Tigernut milk (6.35). The addition of leaf extracts reduced the pH of the tigernut beverages. The pH values observed in this study were lower and also lower than the pH of tigernut milk flavored with *Moringa oleifera* leaf extracts, as reported by [7]. The extremely low pH of carbonated soda and energy drinks (ranging from pH 2.6 to 2.98) has been identified as a key factor in tooth erosion [51]. The pH values of the extracts and tigernut beverages in this study ranged between 5.50 and 6.46, which were significantly higher than those reported for carbonated beverages.

3.6. Total Phenolic and Flavonoids Content of the Extracts and Beverages

Polyphenols are secondary plant metabolites abundant in plant-based foods. Figure 3a illustrates the total phenolic content of the extracts and formulated beverages. The total phenolic content of the extracts ranged from 0.39 to 1.09 mg/100 ml, while that of the beverages ranged from 0.13 to 0.62 mg/100 ml. This indicates that Soursop extract (0.96 mg/100 ml) had the highest phenolic content

among the extracts, whereas tigernut milk with *Morinda lucida*, miracle leave and sour sop extract had the highest phenolic content among the beverages. Plants contain antioxidants that help prevent free radical damage and reduce the risk of chronic diseases [52]. There were significant differences ($P < 0.05$) between the extracts and beverages. The phenolic content observed in this study was lower than those reported for gluten-free acha and tigernut beverages [28] and for tigernut fortified with *Vernonia amygdalina* and *Momordica charantia* [7].

The flavonoid content of the extracts and beverages ranged from 0.15 to 0.96 mg/100 ml and 0.11 to 0.36 mg/100 ml, respectively (Fig. 3b). Soursop extract (0.96 mg/100 ml) had the highest total flavonoid content among the extracts. Among the beverages, tigernut milk with *Morinda lucida*, miracle leave and sour sop extract (0.36 mg/100 ml) had the highest flavonoid content, indicating that the inclusion of soursop, *Morinda lucida*, and miracle leaf extracts significantly ($P < 0.05$) enhanced the total flavonoid content. Leafy vegetables are rich sources of phenolic and flavonoid compounds [53, 54]. The presence of flavonoids in fortified beverages is desirable due to their antioxidative properties, which function through various mechanisms, including free radical scavenging, metal ion chelation, and inhibition of free radical producing enzymes [55, 7]

3.7 Antioxidants activities of Extracts and Beverages

The antioxidant activities of the extract's and formulated beverages were evaluated with two complementary assays: DPPH and FRAP. When DPPH reacts with food products, it decolorizes from purple to yellow, indicating the presence of a proton radical scavenger. Figure 4a shows the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, the DPPH of the extracts ranged between 15.58 to 51.63% and beverages ranged from 82.71 to 86.24%. Beverages fortified with leaves extracts have higher DPPH radical scavenging activity, result also shows that soursop extract (51.63%) has the highest value of DPPH and Tigernut milk with soursop extract (86.24%) has the highest DPPH content among the beverages sample. [54, 7] reported the polar extract of leafy vegetables are better scavengers of DPPH than non-polar extracts.

Figure 4b shows the ferric reducing antioxidant power (FRAP) of extracts and beverages, FRAP content of the extracts varied from 0.10 to 0.93%, whereas the beverages ranged from 6.20 to 15.06%. The results show the FRAP assay, which assesses the reducing capacity of antioxidants in extracts and beverages that react with the ferric tripyridyltriazine (Fe-TPTZ) complex to produce blue-coloured ferrous tripyridyltriazine (Fe-TPTZ). The study found that tigernut beverages had high ferric reducing potential, possibly due to their low pH, since FE-TPTZ is reduced to FE-TPT. Soursop extract (15.06%) showed the highest ferric-reducing antioxidant activity among all prepared beverages. This is comparable to the published FRAP levels for beverages including fruit juice and plant-based milk [56].

3.8. Anti-diabetic Properties of the Extracts and Beverages

Medicinal plants with antidiabetic properties are beneficial in the treatment of diabetes. The appropriate regulation of metabolic activities involving key enzymes has been linked to the etiology of type 2 diabetes. By inhibiting these enzymes (α -amylase and α -glucosidase), the digestion and absorption of

carbohydrates are slowed, leading to improved glycemic control. This approach is supported by various studies, including those by [57, 58]. Pancreatic α -amylase and intestinal α -glucosidase play crucial roles in carbohydrate breakdown and absorption in the body [59]. Therefore, their metabolic activity needs to be properly regulated to maintain stable blood glucose levels [60].

Figure 5 indicate the α -amylase and α -glucosidase inhibition of the extracts and formulated beverages. The α -amylase inhibition of the extracts ranged from 25.69% to 38.19%, while that of the tigernut beverages ranged from 10.81% to 60.41%. Similarly, α -glucosidase inhibition in the extracts ranged from 14.65% to 14.89%, whereas in the beverage blends, it ranged from 16.38% to 17.56%. There were significant differences between the samples.

Among the extracts, *Morinda lucida* extract exhibited the lowest α -amylase inhibition (25.69%), whereas Miracle leaf extract showed the highest inhibition (38.19%). A significant difference ($P < 0.05$) in α -amylase inhibition was observed among the extracts. The α -amylase inhibition of the beverages fortified with extracts showed that 100% Tigernut milk had the lowest inhibition (10.81%), whereas Tigernut with soursop extract exhibited the highest inhibition (60.41%). The addition of leafy vegetable extracts significantly inhibited α -amylase in all samples ($P < 0.05$). Tigernut milk fortified with soursop exhibited the strongest α -amylase inhibition, followed by Tigernut with *morinda lucida* and tigernut milk with miracle leaf. The high α -amylase inhibition suggests that these beverages may serve as potential antidiabetic agents with no adverse side effects, making them suitable as dietary options for individuals with non-insulin-dependent diabetes mellitus (NIDDM).

Similarly, α -glucosidase inhibition was observed in all extract samples, with miracle leaf extract showing the highest inhibition (14.89%). The α -glucosidase inhibition of the prepared beverages revealed that Tigernut with soursop extract had the lowest inhibition, whereas tigernut with *morinda lucida*, miracle leaf and soursop exhibited the highest inhibition (17.56%). The presence of plant extracts in the beverages effectively prevented α -glucosidase from breaking down polysaccharides and disaccharides, enhancing their inhibitory efficacy. The inhibition of these enzymes (α -amylase and α -glucosidase) may be attributed to the phenolic and flavonoid content of the beverages [7]. Enzyme inhibition is a promising strategy for diabetes management, as it can significantly reduce postprandial glucose levels [61, 57]

3.9. Sensory Evaluation

The extracts and formulated beverages were evaluated for appearance, colour, taste, mouthfeel, and overall acceptability (Table 6). Samples TGN, TGNSSSE, and TGNMLE received the highest scores for taste, flavour, colour, and overall acceptability. The high sensory ratings for sample TGN may be due to the panellists' familiarity with the product, while the scores for TGNSSSE and TGNMLE may have been influenced by the naturally mild taste of soursop and miracle leaf. Soursop has a naturally sweet, non-bitter taste and is known for its health benefits, including its high vitamin C content, antioxidant properties, and immune-boosting effects. Miracle leaf is also a non-bitter leaf with anti-inflammatory and antidiabetic properties, as well as phenolic compounds that contribute to its health benefits. In contrast, sample TGNMME received the lowest ratings for taste, mouthfeel, and overall acceptability due to its

bitter taste. This bitterness is attributed to the presence of anthraquinone compounds. Similarly, samples TGNMME and TGNMMS exhibited lower sensory attributes compared to the other samples.

4.0 Conclusion

The study revealed the complementary benefits of combining leafy vegetable extracts with tigernut milk drinks in the formulation of functional beverages. The inclusion of leaf extracts in tigernut drinks improved the proximate composition, increased vitamin content (A, B₁, B₃, and C), and enhanced mineral levels, particularly calcium, sodium, and potassium, with *Morinda lucida* contributing significantly. Soursop extract improved the antioxidant properties of the beverages, providing good radical scavenging potential, which helps protect against free radicals and which may retard the progression of chronic diseases. Miracle leaf extract exhibited strong alpha-amylase inhibition, enhancing the antidiabetic potential of the tigernut beverages without adverse effects, making it suitable for non-insulin-dependent diabetes. The inclusion of extracts also inhibited alpha-glucosidase activity, preventing carbohydrate breakdown. Sensory evaluation revealed that tigernut beverages blended with soursop extract were the most preferred in terms of colour, taste, mouthfeel, and overall acceptability. This study has opened up new product for the application of tigernut and leaf extract in production of beverages with antioxidant, alpha-amylase and glucosidase inhibition which enhanced antidiabetic inhibition and enhanced nutritional and functional profile of beverages.

Declarations

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CRedit authorship contribution statement

Sunday Samuel Sobowale: Conceptualization, project administration, visualization methodology, software and writing of the original draft, reviewing, and editing of the manuscript. **Bukola Funmi Olatunji:** Data curation, formal analysis, investigation, methodology, writing the original draft, reviewing, and editing of the manuscript.

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Compliance with ethical standards

Institutional Review Board Statement: The study was conducted in accordance with the institutional guideline. The study procedure was reviewed and approved by the Mountain Top University Centre for

Research, Innovation and Collaboration Ethics Committee, Nigeria (MTU/470/CRIC/01/2025/05). All necessary ethical guidelines were followed to ensure the protection of participants' rights. Confidentiality and anonymity were strictly maintained, with only de-identified data used for analysis.

Ethics and Consent to Participate

The study was conducted in accordance with relevant local and national guidelines and regulations governing the collection and use of plant materials for scientific research. The leaves of *Morinda lucida*, *Annona muricata* (soursop), and miracle leaf ([insert botanical name]) used in this study were collected from plants growing within the local neighbourhood. Their collection and utilisation were carried out responsibly and in compliance with applicable guidelines. The plant materials were obtained with due consideration for environmental sustainability and were used exclusively for research purposes. No protected or endangered plant species were involved in the study. No specific collection permit or licence was required for the collection of these plant materials.

Ethics and Consent to Participate

The plant materials used in this study, comprising the leaves of *Morinda lucida*, *Annona muricata* (soursop), and miracle leaf, were collected from wild plants growing along roadsides in Bioku, Iyana-Agbala, and Adeggbayi, Ibadan, Oyo State, Nigeria. The plants were identified and their identities confirmed by a staff member of the Department of Horticulture at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The collection and use of the plant materials were conducted in accordance with applicable local and national guidelines and regulations governing plant collection for scientific research. The plant materials were collected exclusively for research purposes, with due consideration for environmental sustainability and the responsible utilisation of plant resources.

Consent to participate

The objectives of the study were thoroughly explained to all participating involve in the tigernut beverages before obtaining informed consent. Participants were fully informed on the local name of plants (soursop, *Morinda lucida* and miracle leaf) used in the fortification of tigernut beverages and the significance of the study, purpose, procedures, and implications. Though, the plants materials were wild without permission from the local streets of Bioku, Iyana-Agbala, Adeggbayi, while tigernuts were purchased from Gbagi Market in Ibadan, Oyo State, Nigeria. Each participant voluntarily agreed to take part in the sensory evaluation after understanding their right to withdraw at any stage without consequences. Informed consent was obtained verbally and in writing to ensure that participants are fully aware of their involvement.

Consent for publication

Not Applicable.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors (s) declare that no competing of interest exist in the submitted manuscript, and all authors have approved it for publication.

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Tables

Tables 1-5 are available in the Supplementary Files section.

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Figures

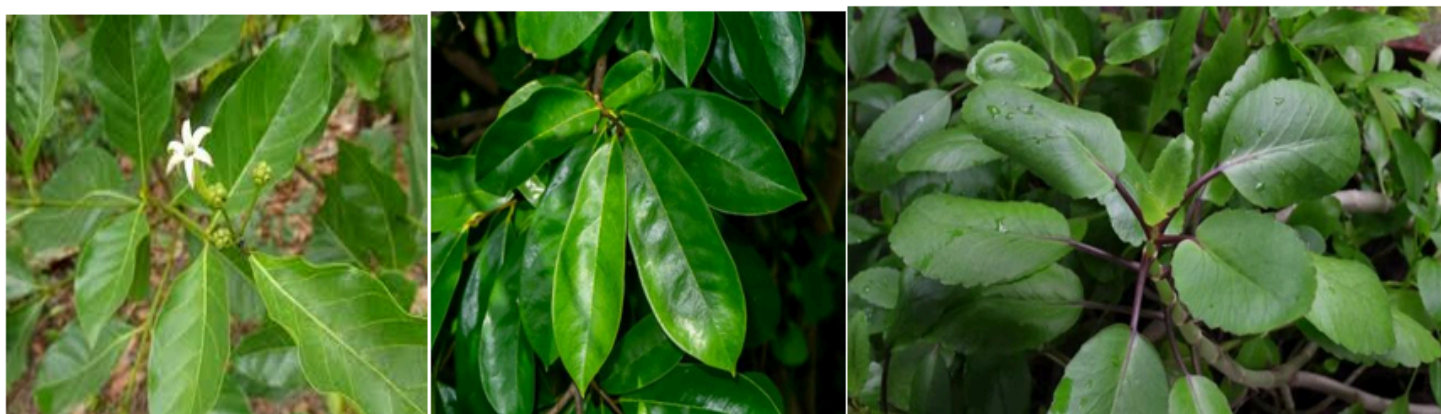


Figure 1

Figure 1a,b&c: (a) Pictorial diagram of Morinda Lucida Plant, (b) Soursop plant and (c) Miracle Leaf

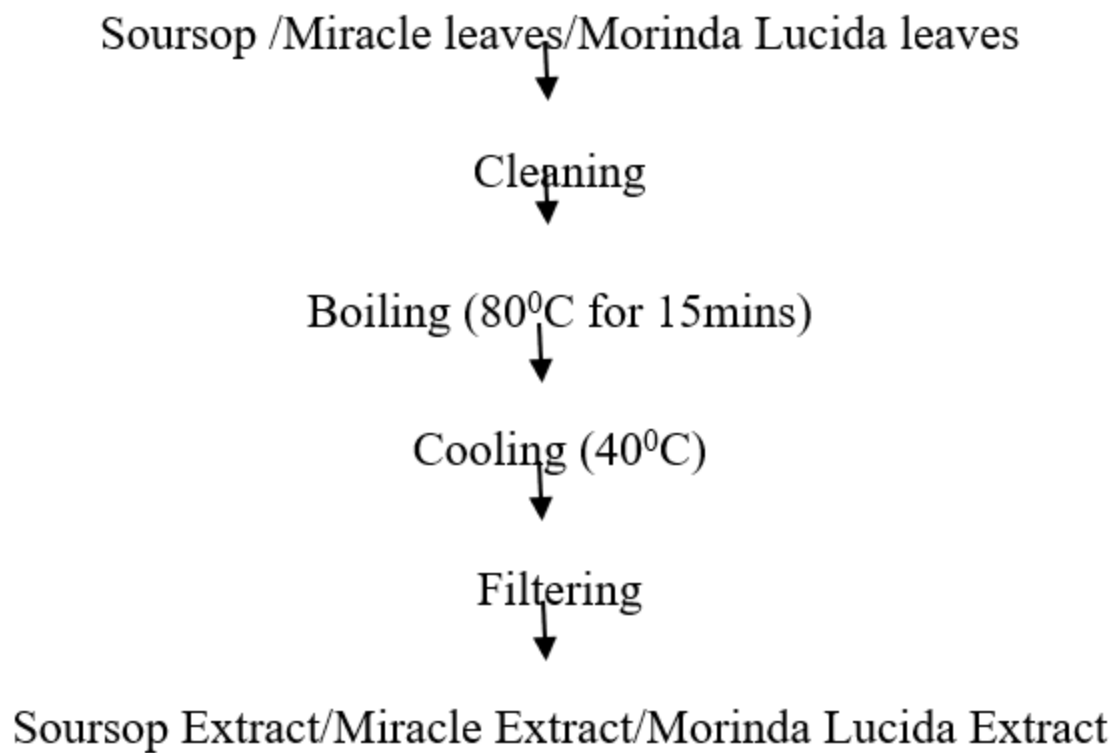


Figure 2

Figure 2a: Processing of leaves extracts

Source: (7)

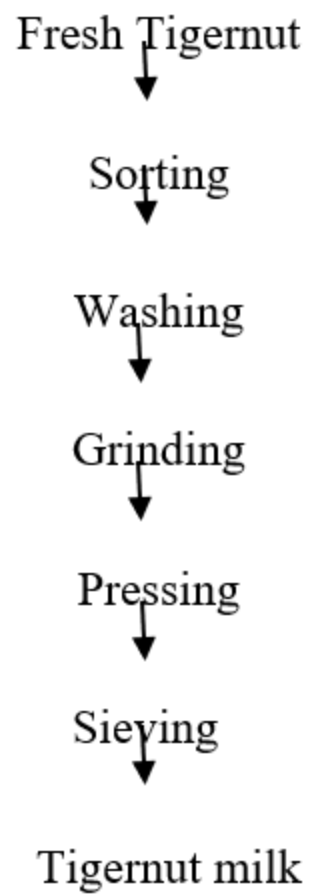


Figure 3

Figure 2b: Processing of tigernut milk

Source: (7)

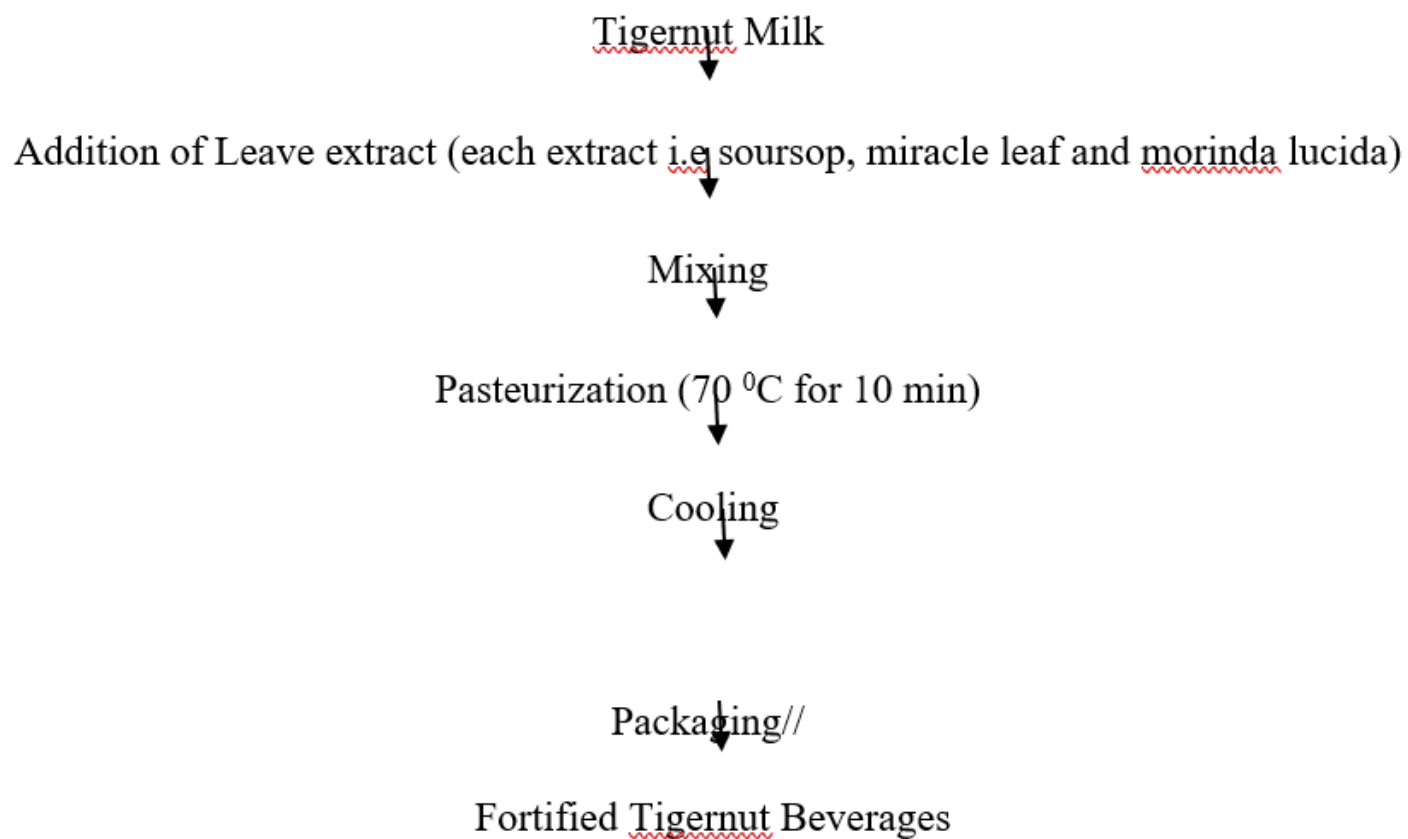


Figure 4

Figure 2c: Processing of fortified tigernut beverages

Source: (7)

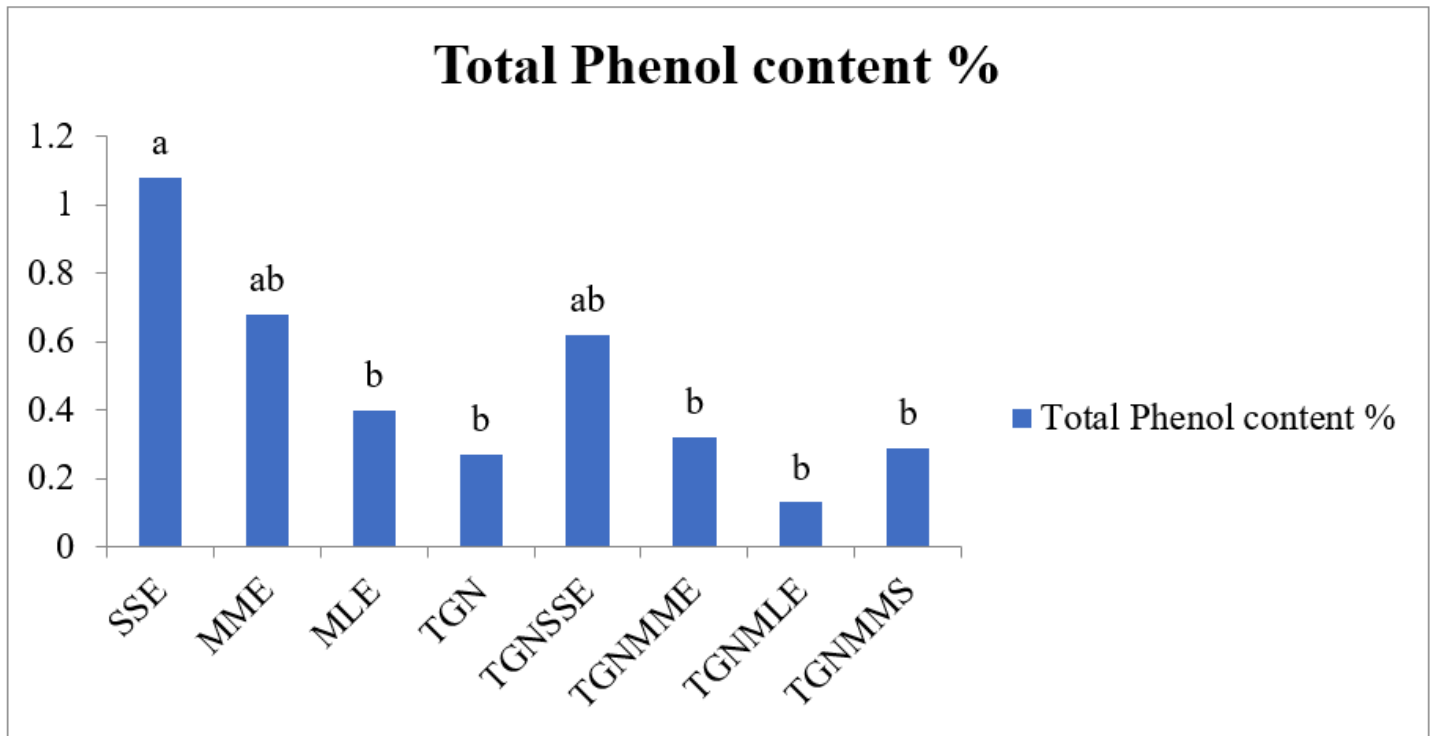


Figure 5

Figure 3a: Total phenolic content of leaves extracts and tigernut beverages fortified with extracts

Values are mean $\pm$ standard deviation of triplicate determinations. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). SSE - 100% Soursop Extract; MME - 100% Morinda Lucida Extract; MLE - 100% Miracle Leaf extract; TGN - 100% tigernut; TGN SSE - 85:15 (tigernut milk: Soursop extract); TGNMME - 85:15 (tigernut milk: Morinda Lucida extract); TGNMLE - 85:15 (tigernut milk: Miracle leaf extract); TGNMMS - 85: 5:5:5 (Tigernut milk: Soursop: Morinda lucida: Miracle leaf extract)

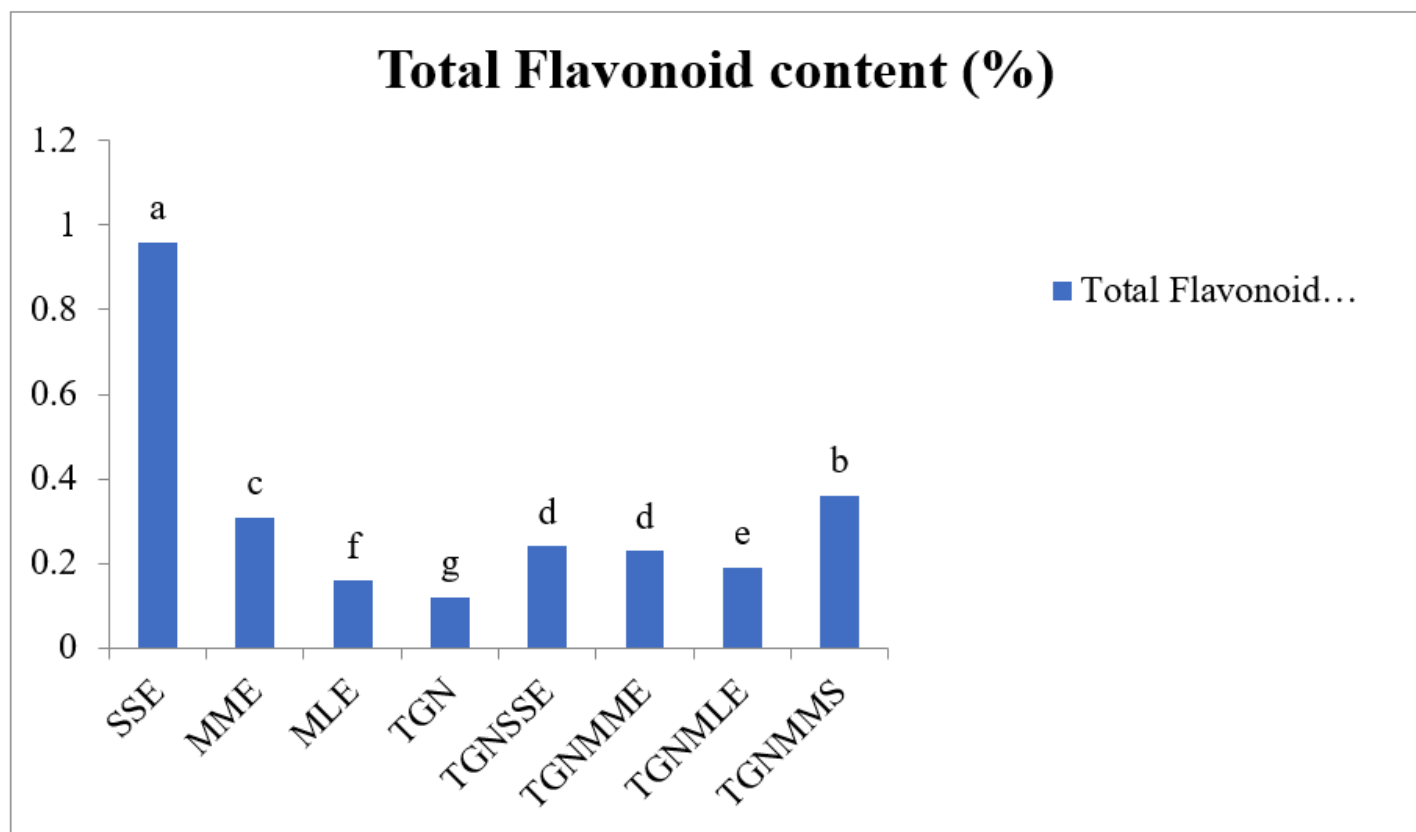


Figure 6

Figure 3b: Total flavonoid content of leaves extracts and tigernut beverages fortified with extracts

Values are mean $\pm$ standard deviation of triplicate determinations. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). SSE - 100% Soursop Extract; MME - 100% Morinda Lucida Extract; MLE - 100% Miracle Leaf extract; TGN - 100% tigernut; TGNSSE - 85:15 (tigernut milk: Soursop extract); TGNMME - 85:15 (tigernut milk: Morinda Lucida extract); TGNMLE - 85:15 (tigernut milk: Miracle leaf extract); TGNMMS - 85: 5:5:5 (Tigernut milk: Soursop: Morinda lucida: Miracle leaf extract)

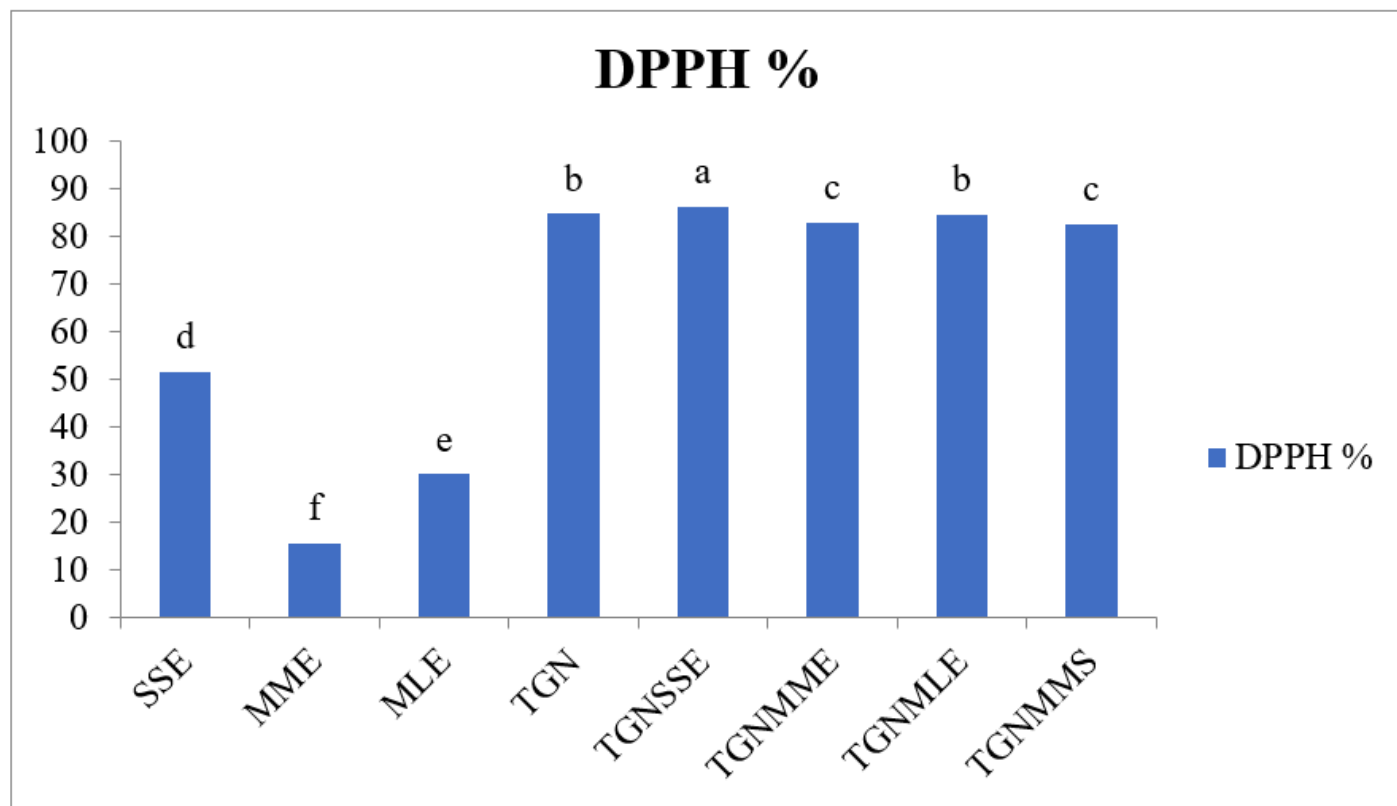


Figure 7

Figure 4a: DPPH of leaves extracts and tigernut beverages fortified with extracts

Values are mean $\pm$ standard deviation of triplicate determinations. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). SSE - 100% Soursop Extract; MME - 100% Morinda Lucida Extract; MLE - 100% Miracle Leaf extract; TGN - 100% tigernut; TGNSSSE - 85:15 (tigernut milk: Soursop extract); TGNMME - 85:15 (tigernut milk: Morinda Lucida extract); TGNMLE - 85:15 (tigernut milk: Miracle leaf extract); TGNMMS - 85: 5:5:5 (Tigernut milk: Soursop: Morinda lucida: Miracle leaf extract)

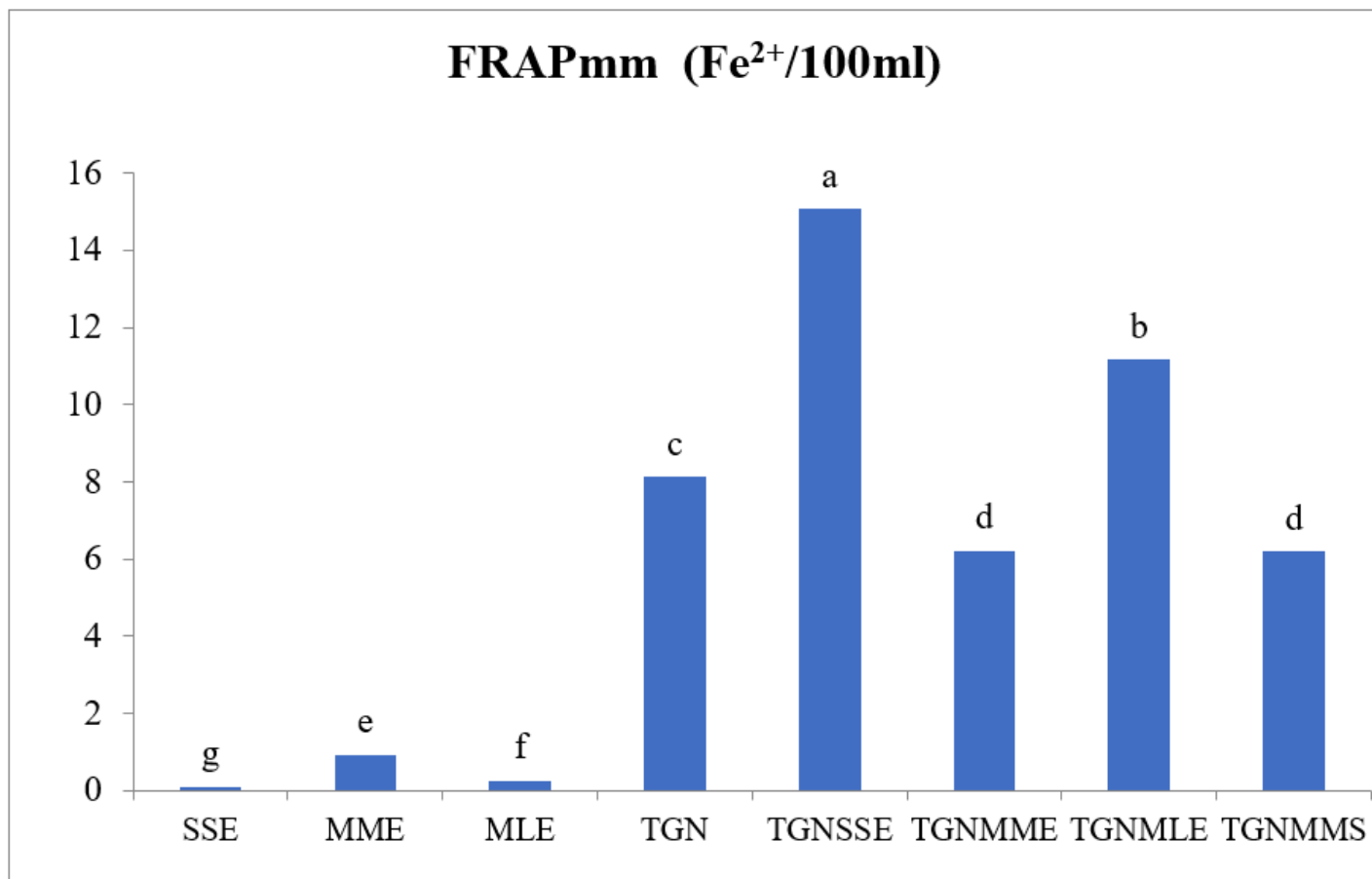


Figure 8

Figure 4b: FRAP of leaves extracts and tigernut beverages fortified with extracts

Values are mean $\pm$ standard deviation of triplicate determinations. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). SSE - 100% Soursop Extract; MME - 100% Morinda Lucida Extract; MLE - 100% Miracle Leaf extract; TGN - 100% tigernut; TGN SSE - 85:15 (tigernut milk: Soursop extract); TGN MME - 85:15 (tigernut milk: Morinda Lucida extract); TGN MLE - 85:15 (tigernut milk: Miracle leaf extract); TGN MMS - 85: 5:5:5 (Tigernut milk: Soursop: Morinda lucida: Miracle leaf extract)

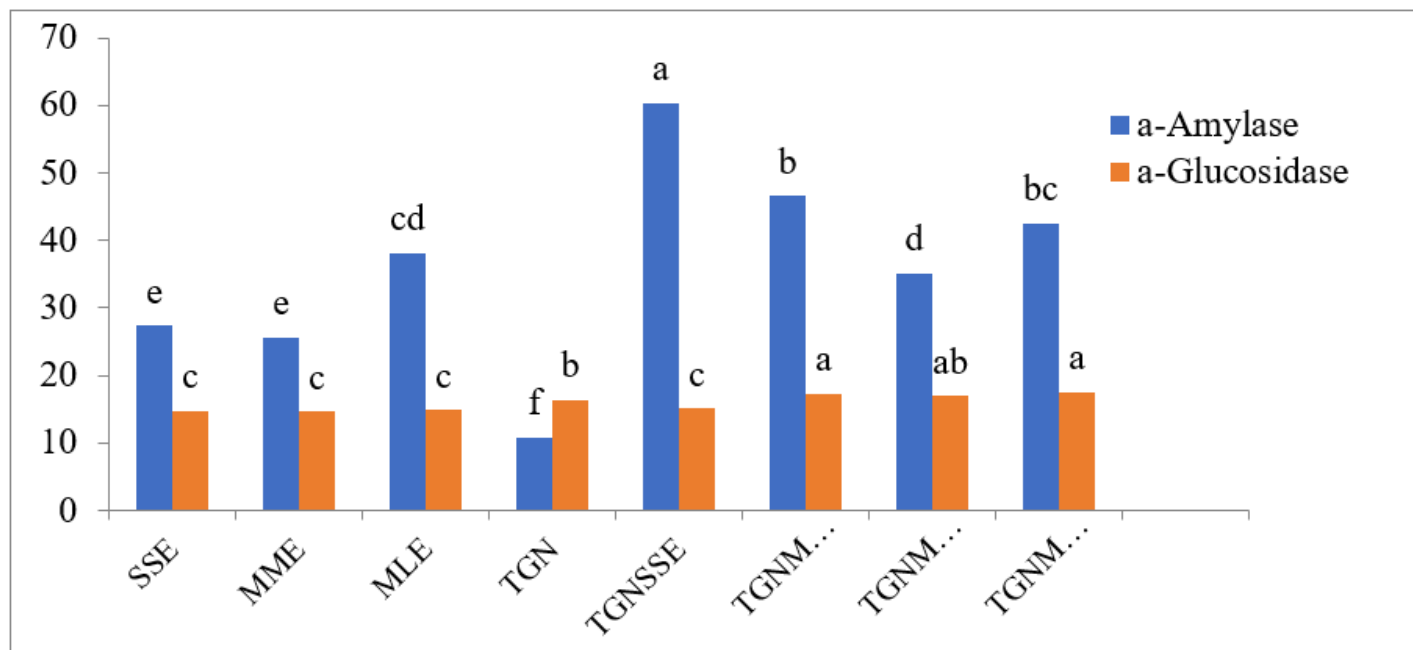


Figure 9

Figure 5: a-Amylase and a-Glucosidase of leaves extracts and tigernut beverages fortified with extracts

Values are mean $\pm$ standard deviation of triplicate determinations. Means on the same column with different sets of superscripts are statistically different ($p \leq 0.05$). SSE - 100% Soursop Extract; MME - 100% Morinda Lucida Extract; MLE - 100% Miracle Leaf extract; TGN - 100% tigernut; TGNSE - 85:15 (tigernut milk: Soursop extract); TGNMME - 85:15 (tigernut milk: Morinda Lucida extract); TGNMLE - 85:15 (tigernut milk: Miracle leaf extract); TGNMMS - 85: 5:5:5 (Tigernut milk: Soursop: Morinda lucida: Miracle leaf extract)

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