Original Research Article

Quality evaluation of tea brewed from blends of soursop (*Annona muricata*) and moringa (*Moringa oleifera*) leaves.

.ABSTRACT

Tea is commonly made from the leaves of *Camellia sinensis*. Production of similar drinks from other plant leaves with potential health benefits would help to prevent diseases. This study examined the chemical composition and antioxidant activity of tea made from blends of dried moringa (*Moringa oleifera*) and soursop (*Annona muricata*) leaves. Mature, fresh and green leaves from both plants were washed in water and sun-dried for 10 h. The dried leaves were milled and sieved to obtain the tea powders. Blends of soursop:moringa tea were formulated as follows: A:100% Soursop, B: 100% Moringa, and soursop:moringa blends as C:50:50%; D: 60:40% and E: 40:60%. Ten grams of each blend of tea powder was brewed in 100 ml of hot water (90^{o}C) for 10 min and cooled to room temperature (28 ±.2^{o}C) before analysis. From the result, 50:50 soursop-moringa tea gave the highest levels of vitamins C and A. Mineral levels were significantly different among the samples ($p<0.05$) with higher values recorded for calcium (2117.10 mg/100ml) sodium (146.02 mg/100ml), magnesium (362.03 mg/100ml), phosphorous (241 mg/100ml), zinc (7.13 mg/100ml) and potassium (1207.20 mg/100ml) in 50:50 soursop-moringa tea. The pH differed significantly ($p<0.05$) in all the tea samples and ranged from 7.28–7.81. Total solids gave values ranging from 3.47mg/l-3.82mg/l ($p<0.05$) and total sugars 1.12–3.07% ($p<0.05$ The amount of tannin was significantly higher ($p<0.05$) in all tea blends compared to other antinutrients analyzed in this study and ranged from 8.95-9.84%. Assessment of the antioxidant capacity by Diphenol-2,2picrylhydroxyl (DPPH) and Ferric reducing antioxidant power (FRAP) showed significant differences ($p<0.05$) among the tea samples with the 50:50 soursop:moringa blend having the highest antioxidant activity with values up to 89.04 % and 531.44 (µM/L) in each case. Overall the soursop-moringa tea blends exhibited good chemical composition and antioxidant activity, with 50:50 formulation showing the best nutritional quality attributes.

Keywords: [soursop (Annona muricata), moringa (Moringa oleifera), tea, antioxidant activity, DPPH, FRAP]

1 INTRODUCTION

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the *Camellia sinensis,* an evergreen shrub native to Asia [1]. It is one of the most widely consumed drinks in the world. There are different kinds of tea which include the Chinese green tea with a cooling, slightly bitter and astringent flavor and others (black tea, and oolong tea) having different sweet, nutty, floral or grassy notes [2]. The different varieties of tea undergo different processing steps resulting in their unique quality characteristics. Tea contains caffeine, vitamins, minerals, and polyphenols, and has been found to prevent diseases such as cancer and obesity [3][4]. Bioactive flavonoids in green and black teas showed high antioxidant activity [5].The consumption of three or more cups of green tea daily reduced the risk of heart attack [6]. Although tea is known to come from the tea plant; *Camellia sinensis,* today there are various sources of such drink made from other plant leaves. Soursop (*Annona muricata*) is a tropical plant belonging to the *Annonaceae* family. The plant is widely promoted for its health benefits [7]. Soursop leaf extract is very good for the prevention of various diseases such as cancer through the inhibition of cancer cell growth [8]. Other benefits include the ability to boost the immune system, improve digestion and reduce inflammation. The leaf contains calcium, potassium, iron, vitamins A, B and C, lipids, stearic acid, gentisic acid, and annomuricin A, B, C, and E [9]. Moringa, which

is of the flowering plant family *Moringaceae,* is native to parts of Africa and Asia and the most widely cultivated specie is Moringa *oleifera*. It contains a significant amount of vitamins A, C and E; calcium, potassium, and protein [10]. Moringa leaves, flowers and seeds contain antioxidants like flavonoids, polyphenols and ascorbic acid. The leaf extract has high antioxidant activity free-radical scavenging capacity and inhibition of lipid, protein and DNA oxidation [11]. This prevents the damage and degradation of cells caused by free radicals in the body. Moringa also confers health benefits such as reducing some diabetes symptoms, protecting the cardiovascular system and the liver, supporting brain health, exhibiting antimicrobial and antibacterial properties [12]. The prevalence of chronic non-communicable diseases such as cancer, cardiovascular diseases and high blood pressure in developing countries has been a great concern. The formulation of food products such as tea and other healthy beverages with significant nutrients to alleviate and prevent these diseases is of great importance. Therefore this study aims to evaluate the chemical composition and antioxidant activity of tea made from blends of soursop and moringa leaves.

2 MATERIAL AND METHODS

2.1 Preparation of Samples

Mature, fresh and green soursop and moringa leaves were washed in tap water and sun-dried (10 h) on an elevated platform. The dried leaves were milled to obtain the tea powders using a blender for 5 min and sieved respectively, with 355 microns sieve. Blends of soursop:moringa tea powders were formulated as follows: I:100% Soursop (100:0%), II: 100% Moringa (0:100%), and soursop:moringa blends (III:60:40%:IV:50:50%;and V: 40:60%). The total weight of tea powder was based on 10 g. Each formulation (10 g) was brewed in 100 ml of hot water (90^{o}C) for 10 min. The tea extract was cooled to room temperature (28±2^{o}C) before the analysis. All samples were formulated and analyzed in triplicates.

2.2 Chemical Analysis

2.2.1 Chemical composition

2.2.1.1 Determination of minerals

Calcium and magnesium were analyzed using Versanate EDTA complexiometric titration described by [13]. Tea extract (20 ml) was mixed with 20 ml of ammonia buffer to adjust the pH to 10.0. One milliliter of Eriochrome black T indicator (0.2 g of indicator in 15 ml of concentrated ammonia solution and 5 ml absolute ethanol) was added to the mixture. The mixture was titrated with 0.02 N EDTA solution until a permanent deep blue colour appeared. A reagent blank (control), calcium and magnesium standard solutions were also titrated with 0.02N EDTA solution. Each titration was repeated three times. Standard curves of Ca and Mg concentrations were plotted with the amount EDTA used for each titration. Calcium and magnesium levels were estimated by extrapolation using the standard curve.

Potassium and sodium were determined using the flame photometry method. Five milliliters of 3 M HCL was added to 50 ml of tea extract. The mixture was digested at boiling temperature and evaporated to reduce the volume to 20 ml. The emission intensities of the samples and standard potassium and sodium solutions were recorded. The experiment was repeated two more times and the mean values of concentration of the standards were plotted against their emission intensities respectively. Concentrations of K ad Na in the samples were estimated by extrapolation using the standard curve in each case [14].

Phosphorus was determined using the Molybdovandate method described [13].Samples were prepared by digesting 10 g of tea extract with 5 ml of concentrated HNO_3 and 1 ml of $HClO_4$ at 100 ^{o}C in a closed Teflon cup for 5 h to obtain a clear solution. The solution was cooled and diluted to 50 ml with deionized water. Ten milliliters of 6N HNO_3 was added to 5

ml of the test sample and 10 ml of 0.1 mg/ml phosphorus standard solution (KH_2PO_4) in 100 ml volumetric flasks, respectively. Ten milliliters of 0.25% ammonium monovanadate and 10 ml of 5% ammonium molybdate were added to the flasks and diluted to volume with deionized water. The solutions were mixed thoroughly and allowed to stand for 15 min for complete colour development. The absorbance of each solution was measured in a spectrophotometer in a 1 cm cell at 400 nm using a reagent blank for autozero. The concentration of phosphorus was calculated using the formula:

$$P\ (mg/100\ ml) = \frac{Absorbance\ of\ sample\ \times 1\ \times total\ volume\ (ml) \times 100}{Absorbance\ of\ standard\ \times volume\ of\ dluted\ sample\ \times weight\ of\ sample}$$

Iron was determined colorimetric method of [13] Samples were wet-digested HNO_3 and 1 ml of $HClO_4$ and diluted to 50 ml volume with deionized water. Standard solutions were prepared from ferrous ammonium sulphate. Emission intensities of samples and standard solutions were determined at 508 nm. A standard curve was prepared and used to estimate the amount of iron in the test sample.

Zinc was determined by atomic absorption spectroscopy. Tea extract (10 g) was digested with 5 ml of conc. HNO_3 and 1 ml of $HClO_4$ at 100 ^oC. The solution as diluted to 50 ml and absorbance was read at 214 nm. The concentration of zinc was calculated from a standard calibration curve and expressed as mg per 100 ml of tea extract [13].

2.2.1.2 Determination of vitamins

Beta carotene was determined by the spectrophotometric method. Five milliliters of the sample was mixed with 30 ml of 95% ethanol and allowed to stand for 20 min at 70-80^oC in a water bath with periodic mixing. The solution was cooled rapidly with running water and filtered. The volume of the filtrate was recorded and 30 ml) distilled water was added to it. The solution was separated with three portions of 25 ml of ether (re-extracting the bottom layer in each case) in a separating funnel. The ether extract was transferred to a separating funnel and washed with 50 ml of distilled water. The extract was evaporated to dryness and the residue was reconstituted with 10 ml of isopropyl alcohol. Absorbance was recorded at 436 nm for the extract and standards and the concentration of beta carotene was calculated from a calibration curve [13].

Vitamin C was determined by the titrimetric method described by [13]. Tea extract (10 g) was weighed into a 100 ml volumetric flask and adjusted to a pH of approx. 1.2 with $HPO_3.CH_3COOH.H_2SO_4$ solution. The solution was diluted to 100 ml volume with $HPO_3.CH_3COOH$. Five milliliters of the sample, ascorbic acid standard, and reagent blank were respectively titrated with indophenol solution (2,6-Dichloroindophenol (50 mg) and 42 mg of NaHCO3 dissolved in water in 200 ml volumetric flask and made up to volume with water. The ascorbic acid standard (ranging from 0.2-1.0 mg/ml) was used to plot the standard curve and Vitamin C was expressed as mg/100 ml of sample.

2.2.1.3 Hydrogen ion concentration (pH)

The pH of tea samples was determined using a standard simple glass electrode pH meter as recommended by [13].

2.2.1.4 Total Phenolic content

Total phenol was determined using the Folin Ciocalteu reagent colorimetric method. Samples or (0.1 ml) were transferred into test tubes containing 6.0 ml of distilled water. Undiluted Folin-Ciocalteu reagent (0.5 ml) (F-9252, Sigma) and 1.5 ml (200g/l) of saturated

sodium carbonate (Sigma, Aldrich) were added one at a time to the mixture. The total volume was made up to 10 ml with distilled water. The solutions were vortexed and incubated at 40^{o}C for 2 h in a water bath. Absorbance was determined at 765 nm using Ultrospec 2000 - spectrophotometer (Pharmacia Biotech.UK) with reagent blank. Phenolic acid levels were estimated as µg gallic acid equivalent per ml of the sample [15].

2.2.1.5 Flavonoid

Flavonoid determined using the method of [16]. Sample (5 ml) was added to 50 ml of 2N HCL solution at room temperature and boiled for 30 min in a water bath. The solution was allowed to cool and then filtered using Whatman filter paper No 40. Ethyl acetate was added dropwise (approx. 25 ml) to the filtrate. The residue recovered after filtration was dried in the oven at 100^{o}C for 30 min. Flavonoid was calculated as a percentage of the sample weight.

2.2.1.6 Saponin

Saponin determination was carried out according to the method of [16]. Five milliliters of the sample was added to 50 ml of 20% aqueous ethanol and heated with agitation at intervals for 90 min at 55^{o}C in a water bath. It was filtered the extract was concentrated to 40 ml at 90^{0}C. It was transferred to a separating funnel and 40ml diethyl ether was added with vigorous shaking. Separation by partition was done repeatedly until the aqueous layer was seen as clear. Saponin was extracted with 60 ml of N-butanol and the extract was washed with 10 ml of 5% aqueous NaCl solution. It was evaporated to dryness and oven-dried at 60^{0}C to a constant weight. Saponin was expressed as a percentage of the sample weight.

2.2.1.7 Tannin

Tannin was determined using the method of [16]. Five millilitres of the tea extract was added to 12.5 ml of indigo-carmine solution and 375 ml of distilled water. The mixture was titrated against $KMnO_4$ solution ('A' ml) until a faint pink endpoint colour was reached. This volume of $KMnO_4$ solution was used for both tannin and related compounds. To calculate the volume of $KMnO_4$ solution ('B' ml) used for tannin related compounds, 50 ml of tea extract was mixed with 25 ml of gelatin solution, 50 ml of acidic NaCl solution (25 ml of concentrated H2SO4 added to 975 ml of saturated NaCl solution) and 5 g of kaolin powder. The mixture was shaken for 15 min and filtered using Whatman No. 1 filter paper. The filtrate (12.5 ml) was mixed with the same volume (12.5 ml) of an indigo-carmine solution and 375 ml of distilled water. This mixture was then titrated against $KMnO_4$ solution until a faint pink is obtained. The volume of $KMnO_4$ solution used to titrate the true tannin was calculated ('A' – 'B') and the concentration of tannin was estimated using:

$$1\ ml\ of\ standad\ KMnO4\ solution = 0.595\ ml\ of\ 0.1\ N\ oxalic\ acid$$
$$1\ ml\ o\ f\ 0.1\ N\ oxalic\ acid = 0.0042 g\ of\ tannin$$

2.2.1.8 Alkaloid

Alkaloid was determined using the alkaline precipitation method [17]. Sample (2.5g) was mixed with 200 ml of 10% v/w ethanolic acetic acid and allowed to stand for 4h at room temperature. The solution was filtered using a Whatman filter paper No. 40 and concentrated to ¼ their original volume by evaporation in a water bath. Ammonium hydroxide (1%) was added dropwise until precipitation occurred. The mixture was filtered and the precipitate was washed using 20 ml of 1% NH_4OH and then filtered using a Whatman filter paper. The residue was dried in an oven (60^{o}C) until a constant weight was achieved. Alkaloid was expressed as a percentage of the sample weight.

2.2.1.9 Cyanogenic glucoside

Cyanogenic glucoside was determined by the spectrophotometric method as described by [18]. Five milliliters of each sample was mixed with 50 ml of distilled water and kept for 24 h

at 28±2^{o}C. The filtrate (1 ml) was mixed with 4 ml of alkaline picrate and incubated in a water bath at 50^{o}C for 5 min. After colour development absorbance was recorded against a reagent blank at 490 nm. Cyanide levels were calculated from the standard calibration plot of varying concentrations (0.1-0.5 µg/ml) of potassium cyanide.

2.2.1.10 Total solids

Samples (5 ml) were dried in the oven at 105 ^{0}C to a constant weight. The weight of lost moisture was calculated by difference and expressed as a percentage of the sample weight [13].

2.2.1.11 Total sugars

Total sugar was determined using the method of [13]. Ten milliliters of tea extract was mixed with 100 ml of distilled water and centrifuged for 10 min. The supernatant (0.5 ml) was mixed with 5 ml of 4% phenol, 2.5 ml of 96% sulphuric acid (H_2SO_4)' and 1 ml of distilled water. Absorbance was determined at 490 nm against glucose standards using a spectrophotometer. Total sugar content was calculated from the standard curve:

2.2.2 Antioxidant activity

2.2.2.1 Diphenol-2-2-picrylhydroxyl (DPPH) assay

The antioxidant properties of the tea samples were determined using Diphenol-2-2-picrylhydroxyl (DPPH) method as described by [19] with slight modification. One milliliter of each extract was added to 10 ml of methanol. Then the solution was mixed using a vortex and left to stand at room temperature (28 ± 2^{o}C) for 1 h in a dark cupboard. It was stirred and filtered into a clean beaker. One milliliter of the filtrate was transferred into a test tube and left to stand in the dark for 30 min after the addition of 1 ml of DPPH solution. The absorbance of the solution was measured at 517 nm using a spectrophotometer, with the degree of discoloration of the solution indicating the scavenging efficiency of the added substance. The free radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$Free\ radical\ scavenging\ activity = 100 * \ 1 - absorbance\ of \frac{sample}{Absorbance\ of\ reference}$$

2.2.2.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) was determined using the method described by [20]. Three milliliters of prepared FRAP reagent was mixed with 200 µl of the sample in a test tube. A blank sample was also prepared. With deionized water. The absorbance of the tea sample was determined at 593 nm against the blank, after 30 min incubation at 37^{o}C. The absorbance of standard solutions of 200, 400, 800, 1200, and 1600 µM prepared from an aqueous solution of FeSO4.H2O was used to plot the calibration curve. FRAP values obtained for the samples were expressed as µM of Fe^{2+} equivalent per litre of tea extract.

2.3 Statistical Analysis

Data obtained from triplicate determinations of the samples were analyzed and significant differences between the samples were tested using Analysis of variance (ANOVA) Duncan's multiple range test with SPSS statistical software (version 20, IBM SPSS, UK).

3 RESULTS AND DISCUSSION

3.1 Chemical Composition

3.1.1 Vitamin and mineral compositions of soursop-moringa tea

The results of vitamin and mineral compositions of soursop-moringa tea blends are shown in Table 1. The level of vitamins in the tea blends was significantly different ($p<.05$). Soursop-moringa tea (50:50%) and 100% moringa tea had the highest (44.22 mg) and lowest (18.41 mg) levels of beta-carotene respectively. Beta carotene is the precursor of vitamin A and is converted to vitamin A by the liver. Vitamin A is important for the maintenance of immune systems, good vision, and skin health. Soursop-moringa tea blends contain appreciable amounts of vitamin A which could help to boost the immune system and improve vision.

Vitamin C (ascorbic acid) ranged from 20.02 - 91.72 mg/100 ml with the highest level found in soursop-moringa tea (50:50%) and the least level in 100% moringa tea. The values obtained for vitamin C in this study vary from the range of values reported by [21] for fresh moringa leaf (213 mg), moringa leaf powder (16.55 mg), green tea (8.25 mg) and black tea (76.40 mg). This variation could be attributed to the processing methods such as steaming of the leaves as used in the present study which gave a slightly higher value of 20.20 mg for 100% moringa tea compared to 16.55 mg reported for the tea powder by the above researchers. Soursop:moringa tea (50:50%) showed the highest level of vitamin C (91.72 mg/100 ml). vitamin C is an important nutrient possessing the antioxidant ability and protects against free radicals [22]. Drinking up to 3 cups of soursop:moringa tea (50:50) with a high content of vitamin C as observed in this study could help to meet the recommended daily intake of 60 mg [23] and protect the body against free radicals.

The compositions of all the minerals tested in the tea samples were significantly different ($p<0.05$). The 100% moringa tea showed significantly higher levels of the minerals than 100% soursop tea. However, the highest level of calcium, sodium, magnesium, phosphorus, zinc, and potassium was observed with the soursop-moringa 50:50% tea blend while the 100% soursop tea had the least values for all the minerals evaluated. Interestingly, calcium and potassium were most abundant in all the tea blends and ranged from approximately 608-2,117 mg/110 ml and 637-1,207 mg/100 ml, respectively.

The availability of calcium in human nutrition has been associated with reduced risk of osteoporosis, hypertension, colon/breast cancer, kidney stones, and obesity/over-weight [24]. Calcium plays an important role in blood clotting, in muscle contraction and enzyme metabolic processes [25]. Lower values of calcium (which ranged from 1.39–6.87mg/100 ml) in moringa tea infusions were reported by [26] than observed in this study. Gabriel and Nkemakonam [21] reported 465.50 - 2057.50 mg/100 ml of calcium in moringa leaves and the tea samples which compares favorably to the values obtained in this study. Interestingly, the amount of calcium detected in this study suggests that the tea blends are suitable to meet the recommended daily allowance of calcium (1000–1300 mg/day) in humans.

Potassium is an important element that helps in the maintenance of acid-base balance in the body and normal functioning of the nervous system [27]. Potassium levels of the tea blends obtained from this study are not surprising since moringa tea leaves have previously been reported to contain an appreciable amount of potassium such as 1349.7 mg/100g [26] and 1845 mg/100g [28]. However, the recommended daily intake of 4000 – 4700 mg/day for potassium [24] could be supplied to the body by drinking up to 400 ml of soursop-moringa tea per day.

The amount of sodium recorded for soursop:moringa tea blends ranged from 101.63 -146.02 mg/100g. Lower values which ranged from 0.63 – 7.86 mg/100mL and 8.13 mg/100g were reported for moringa leaf powder [26][28]. These differences could be attributed to cultivation practices and processing conditions. Sodium plays a variety of important roles in the body such as controlling blood pressure and regulating the function of muscles and nerves, maintaining healthy fluid balance and contributing to proper muscle contraction and nerve impulse conduction [32].

Table 1: Vitamin and mineral composition (mg/100 ml) of soursop:moringa tea blends

Soursop: Moringa tea blends	Beta carotene (µg/100 ml)	Vit.C	Ca	Na	Mg	P	K	Fe	Zn
100:0	36.58^{b}±0.01	88.23^{a}±0.02	608.05^{e}±0.04	101.63^{e}±0.02	157.32^{e}±0.02	187.32^{e}±0.02	637.12^{e}±0.02	8.61^{e}±0.01	3.61^{e}±0.02
0:100	18.41^{e}±0.03	20.02^{d}±0.02	2014.10^{b}±0.02	117.15^{c}±0.01	314.83^{d}±0.02	206.03^{d}±0.12	1041.10^{d}±0.02	14.11^{a}±0.01	6.02^{c}±0.01
60:40	36.49^{b}±0.02	73.92^{e}±0.01	1049.10^{d}±0.02	109.84^{d}±0.01	317.65^{c}±0.02	210.42^{c}±0.02	1100.20^{c}±0.02	9.51^{d}±0.01	4.17^{d}±0.01
50:50	44.22^{a}±0.02	91.72^{b}±0.02	2117.10^{a}±0.03	146.02^{a}±0.02	362.03^{a}±0.02	241.25^{a}±0.02	1207.20^{a}±0.02	12.83^{b}±0.02	7.13^{a}±0.01
40:60	30.12^{c}±0.02	61.65^{c}±0.02	1634.20^{c}±0.02	121.03^{b}±0.02	346.12^{b}±0.02	229.72^{b}±0.01	1184.00^{b}±0.02	11.33^{c}±0.02	6.32^{b}±0.01
‡Green tea	**0.33±0.00	***107.0±0.1	*374.75±19.65	*7.55 ± 0.14	**22.0±0.34	**6.30±0.00	*1703.13±51.5	*20.53±0.76	*3.06±0.12
†RDI (mg)	0.4-1.3	15-120	1000-1300	1200-1500	240-420	700-1250	4500-4700	8-18	8-11

Data are means of triplicate (n=3) analysis ± standard deviation. Means with the same superscripts within each column are not significantly different ($p > .05$). means with different superscript within each column are significantly different ($p<.05$). Tea extract was prepared from 10 g of tea powder in each case. ‡Equivalent values (mg) for regular green tea from *Camellia sinensis*, *[29] **[30] ***[31]. †RDI (Recommended Dietary Intake (mg)) as estimated by the National Academy of Sciences, Food and Nutrition board, USA.

Table 2: Chemical composition of soursop:moringa tea extract

Soursop:moringa tea blends	pH	Total solids (mg/l)	Total sugars (%)	Tannin(%)	Phytate(%)	Alkaloid(%)	Flavonoid(%)	Saponin(%)	Cyanide(%)
100:0	7.28^{e}±0.01	3.82^{a}±0.02	3.07^{e}±0.02	8.95^{e}±0.02	2.52^{e}±0.02	1.93^{e}±0.01	7.34^{a}±0.02	0.25^{e}±0.01	0.22^{c}±0.01
0:100	7.81^{a}±0.01	3.55^{d}±0.02	1.12^{e}±0.01	9.36^{c}±0.02	7.67^{a}±0.03	4.64^{a}±0.02	3.61^{e}±0.12	1.63^{a}±0.02	0.30^{a}±0.01
60:40	7.49^{d}±0.00	3.66^{c}±0.02	2.25^{c}±0.01	9.12^{d}±0.01	4.27^{d}±0.02	2.85^{d}±0.02	6.57^{c}±0.02	0.67^{d}±0.01	0.17^{d}±0.01
50:50	7.73^{b}±0.01	3.72^{b}±0.02	2.36^{b}±0.01	9.84^{a}±0.01	6.17^{c}±0.02	3.93^{c}±0.02	6.83^{b}±0.02	1.31^{b} ±0.02	0.27^{b}±0.01
40:60	7.63^{c}±0.01	3.47^{e}±0.02	2.04^{b}±0.01	9.45^{b}±0.02	6.32^{b}±0.02	4.24^{b}±0.02	4.31^{d}±0.01	0.98^{c}±0.01	0.22^{c}±0.01

Data are means of triplicate (n=3) analysis ± standard deviation. Means with the same superscripts within each column are not significantly different ($p<.05$). Means with different superscript within each column are significantly different ($p<.05$).

The recommended daily allowance of sodium in the human body ranges from 1000 - 1500 mg/day, therefore, daily consumption of 700-1000 ml of 50:50 soursop:moringa tea would help to meet this requirement. Magnesium in the tea samples ranged from 157.32 mg/100mL to 362.03 mg/100mL). This is higher than the level (1.30 – 12.63 mg/100mL) reported for moringa tea infusion and lower than 449.69 mg/100g reported for moringa leaf powder by [26]. Moringa (100%) tea gave a significantly ($p<0.05$) higher level of magnesium than 100% soursop tea. The levels of magnesium recorded in the tea blends suggest a synergistic effect in the release of magnesium in the products as the level of magnesium in the tea samples increased with the increase in the amount of moringa tea powder. Magnesium helps to activate the enzymatic systems responsible for calcium metabolism in bones [33] and forms an essential constituent for reproduction and normal functioning of the nervous system [34][35]. The amount of magnesium recorded in this study suggests the potential of the soursop:moringa tea to supply the recommended daily allowance of magnesium (110 – 300 mg/day) in the body when consumed.

The level of phosphorus observed in soursop:moringa tea followed a similar trend with magnesium and increased with the increase in the amount of moringa tea powder which reflects the levels present in the individual tea powders. The values of phosphorus (187-241 mg/100ml) obtained in this study are within the range of values (74.80 – 225.00 mg/100ml) reported by [21] for moringa tea leaves suggesting high solubility and minimal loss of the mineral after brewing. In this study, the addition of an equal amount of moringa and soursop tea powder gave the highest level of phosphorus. Phosphorus is the second most abundant mineral in the human body and is needed to perform many functions such as assisting in muscle contraction and facilitating nerve conduction [36]. Therefore, the high amount of phosphorus in the soursop-moringa tea products would be very beneficial to health.

Moringa tea leaves are richer in iron compared to soursop as revealed in this study with the highest value obtained for 100% moringa tea. Iron serves as an oxygen carrier in the blood hemoglobin and muscle myoglobin of animals and facilitates the oxidation of carbohydrates, proteins, and fats [37]. It contributes significantly to the prevention of widespread of anemia in developing countries like Nigeria. Soursop:moringa tea samples in this study showed a good amount of iron (8.61 – 14.11 mg/100 ml) and could serve as a good source of non-haem iron to meet the dietary requirement in the body.

Zinc plays important role in the human body such as the synthesis of protein and DNA and boosting of the immune system [37]. It was observed that 100% moringa tea (6.02 mg/100 ml) is richer in zinc compared to 100% soursop tea (3.61 mg/100ml). Higher values have been reported for moringa leaf powder 70.70 mg/100g [26] 148 mg [38] and 858 mg [39]. The low level of zinc reported for the moringa tea extract compared to the leaf powder could be attributed to the effect of processing. Zinc is not stored in the human body and needs to be obtained daily. Interestingly, the value (7.13 mg/100ml) obtained for zinc in 50:50 soursop:moringa tea in this study makes it a good source of zinc for women with recommended daily allowance of 8 mg/day [38].

3.1.2 Hydrogen ion concentration (pH)

The pH of the tea samples ranged from 7.28 – 7.81. This result is within the range of pH (7-10) as reported by [40] for green tea. Soursop-moringa (100%:0%) tea had the least pH value of 7.28, while moringa (100%) tea had the highest value (7.81). The differences in the pH values can be attributed to the difference in the formulation. Hydrogen ion concentration has been reported to be one of the important quality characteristics that describe the stability of bioactive compounds in tea products [41]. Thus high pH values observed in the tea samples suggests a positive effect on the stability of the tea products during storage. Acidic tea such as the black tea has erosive potentials and has been implicated with dental problems [42][40]. Interestingly, soursop:moringa tea blends as observed in this study are not acidic making them suitable for individuals with sensitive teeth.

3.1.3 Total solids

Total solid were significantly ($p<0.05$) different among the tea samples and ranged from 3.47mg/L to 3.82mg/L (Table 2). Total solid is a reflection of solute present in liquid foods. It is inversely proportional to the amount of water present in a liquid food sample. The strength, taste, appearance, and aroma of the tea depend on the level of total solids [43]. Soursop tea (100%) recorded the highest value of 3.82 mg/L while soursop:moringa tea (40:60) recorded the least value (3.47 mg/L). The results obtained in this study showed that the total solid of the tea blends seem to have been affected more by the soursop than the moringa and increased with the increase in soursop leaf powder.

3.2.4 Total sugars

The total sugar content of the tea samples is presented in Table 2. Values obtained for total sugar in soursop:moringa tea blends were significantly different ($p<0.05$) and ranged from 1.12 – 3.07%. Soursop-moringa tea (100:0%) recorded the highest value (3.07%) while soursop-moringa tea (0:100%) recorded the least value (1.12%). Given the low sugar level in moringa, total sugar decreased with an increase in the amount of moringa leaf powder added to the tea blends. The values obtained in this study are considerably low suggesting that soursop-moringa tea products contain less carbohydrate and could be suitable for persons desiring weight loss.

3.2.5 Antinutrients

Antinutrients of tea brewed from blends of dried soursop and moringa leaf powders are presented in Table 2. The result showed significant differences ($p<.05$) in the levels of all the antinutrients among the tea products. Previous studies reported that tea leaves contain anti-nutrients such as saponin, tannins, and phytates [44][45]. However, the concentration of antinutrients could be reduced or eliminated to a tolerable level through processing methods. The tannin content of the tea blends ranged from 8.95 – 9.84% with soursop:moringa tea (50%:50%) having the highest value and 100%soursop tea having the least value. A higher tannin level of 18.90% in *Camellia sinensis* green tea was reported by [33]. The differences observed among the tea products could be linked to the composition of the blends. Astringent flavour is characteristic of tea leaf steeped in hot water due to the presence of catechins and other flavonoids [46]. Tannins can provoke an astringent reaction in the mouth and make the food unpalatable. Tannins also form complexes with proteins in the gut, reducing the digestibility or inhibiting digesting enzymes and microorganisms [18]. The values obtained in this study are above the toxic level reported by [47] for animals (5%) and poultry (0.5 – 7%). To reduce the effect of tannin effect on mineral absorption such as iron, tea could be taken between meals rich in vitamin C or with milk or lemon. However, tea with a high level of tannin has been reported to benefit people with iron overload reducing the need for blood removal. Tannins are good antioxidants and catechin a type of tannin mostly present in tea has been found to decrease total cholesterol, lower blood pressure and minimizes the risk of cancer [47].

Phytic acid forms insoluble salts with essential minerals like calcium, iron, magnesium, and zinc in food, thereby hindering their absorption into the bloodstream [48]. About half the phytic acid phosphorus absorbed into the body is excreted unchanged and is unavailable for utilization [44]. Phytic acid detected in the tea products differed significantly among the tea blends, with 100% moringa tea having the highest level of phytic acid and 100% soursop tea showing the least level. A higher amount of phytic acid (111.76%) was reported by [49] for the regular green tea as compared to the range of 2.52-6.32% observed in this study. It was observed that the amount of phytic acid in the tea blends increased as the quantity of moringa tea powder increased in the blends. Although phytic acid inhibits nutrient absorption it is a good antioxidant. This bioactive compound mediates enzyme activities and regulates vital cellular functions such as cell proliferation and differentiation [50]. Phytic acid has been reported to reduce the incidence of colon cancer when 0.2-0.5% (w/v) of commercial phytic acid was administered in an animal model [51]. Oral administration of Inositol and green tea containing 1% and 2% (w/v) phytic acid reduced colon tumors in rats [52], thus the presence

of phytic acid in moringa tea as observed in this study, makes it a good source for chemoprevention.

Flavonoids are one of the major polyphenols present in tea [53] and are strong antioxidants, also found to be effective antimicrobial substances. They have been reported to possess substantial anti-carcinogenic and anti-mutagenic activities due to their antioxidant and anti-inflammatory properties [54]. The result from this study showed significant differences ($p<.05$) in the level of flavonoid among the tea blends. Soursop tea (100%) had the highest level of flavonoid (7.34%) while the 100% moringa tea had the least amount (3.6%). The addition of soursop tea powder increased the level of flavonoid in the tea blends. However, values obtained from this study (3.61-7.34%) are lower than those (1.62-57.64%) reported for moringa tea infusion by [26]. The variations in the results of the present study could be due to the methods of extraction of polyphenolic compounds, the degree of polarity of the solvents and geographical locations of the plants. Apart from the 100% soursop tea, an equal amount of soursop and moringa tea powders gave an appreciable level of flavonoid suggesting a higher antioxidant potential than the other blends.

The result of saponin ranged from 0.25% to 1.63% and differed significantly ($p<0.05$) among the samples. Moringa tea (100%) had the highest value while 100% soursop tea had the least value. A high level of saponin in moringa tea leaves seems responsible for the high levels detected in the tea blends. According to [55], saponin has immune modulation activities and can regulate cell proliferation as well as health benefits such as anti-carcinogenic effect and cholesterol-lowering activity.

Alkaloids are natural compounds, known to possess antimicrobial properties, due to their ability to intercalate with DNA of microorganisms [56]. Moringa tea (100%) contains the highest level of alkaloids (4.64%) content while 100% soursop tea had the least (1.93%) alkaloid content. Alkaloids in soursop-moringa tea samples showed significantly different ($p<.05$) levels and ranged from 1.93 – 4.64%, which is lower than the value (19.33%) reported by [49]. Some alkaloids exert a stimulating role of the central nervous system such as caffeine. It excites the nervous system, increases heart rate and promotes the elimination of urine. Other alkaloids like theophylline which usually exist in small quantities, aid blood circulation [56]. The alkaloid content of soursop-moringa tea with prolonged consumption in large quantities may generally increase the metabolic activity in the body.

Cyanogenic glucosides are secondary metabolites in foods that contain nitrogen and are capable of releasing toxic hydrogen cyanide when hydrolyzed by an enzyme. It hinders cellular respiration. Toxicity in humans is possible at doses up to 0.5 mg – 3.5 mg per kilogram body weight [57]. The amount of cyanogenic glucoside recorded in this study ranged from 0.17 – 0.30% and there was no significant difference between 100% soursop tea and soursop:moringa tea (40:60%). The level of cyanogenic glycoside decreased with an increase in the amount of soursop leaf powder added to the tea blends. However, detoxification of this compound could be achieved by processing methods such as boiling, drying or fermentation [57]. Thus processing of soursop-moringa leaves to black tea through fermentation would further reduce the level of cyanogenic glycoside in the tea blends.

3.3 Total Phenol and Antioxidant Activity

The total phenolic content in soursop-moringa tea blends ranged from 139.66 – 214.04 mg GAE/L (Fig.1). The determination of the level of total phenolics based on their chemical reducing capacity relative to gallic acid [15]. Tea leaves are good sources of antioxidant compounds that act as free radical scavengers.

A positive relationship between antioxidant activity potential and the amount of phenolic compounds of the crude tea extracts was reported by [58]. The phenol content obtained in this study (139.6 - 214.04 mg/100mL) suggests the potential of the tea products having good antioxidant activity. The highest level of total phenol was observed in 100% moringa tea.

The antioxidant capacity of the tea blends in this study was tested using the ferric reducing antioxidant power assay. This measures the reducing ability of antioxidants against oxidative

effects of reactive oxygen species The reducing effect was most evident with 50% inclusion of both soursop and moringa leaves with FRAP value of 531.44 µM/L (Fig. 1).
When tested with Diphenol-2-2-picrylhydroxyl (DPPH), a similar effect was observed with soursop:moringa tea (50:50%) which showed the highest DPPH value while soursop-moringa tea (100:0%) had the least. This result revealed that tea brewed from moringa leaves has good free radical scavenging activity compared to soursop tea and has the potential of reducing oxidative stress and preventing the onset of diseases.

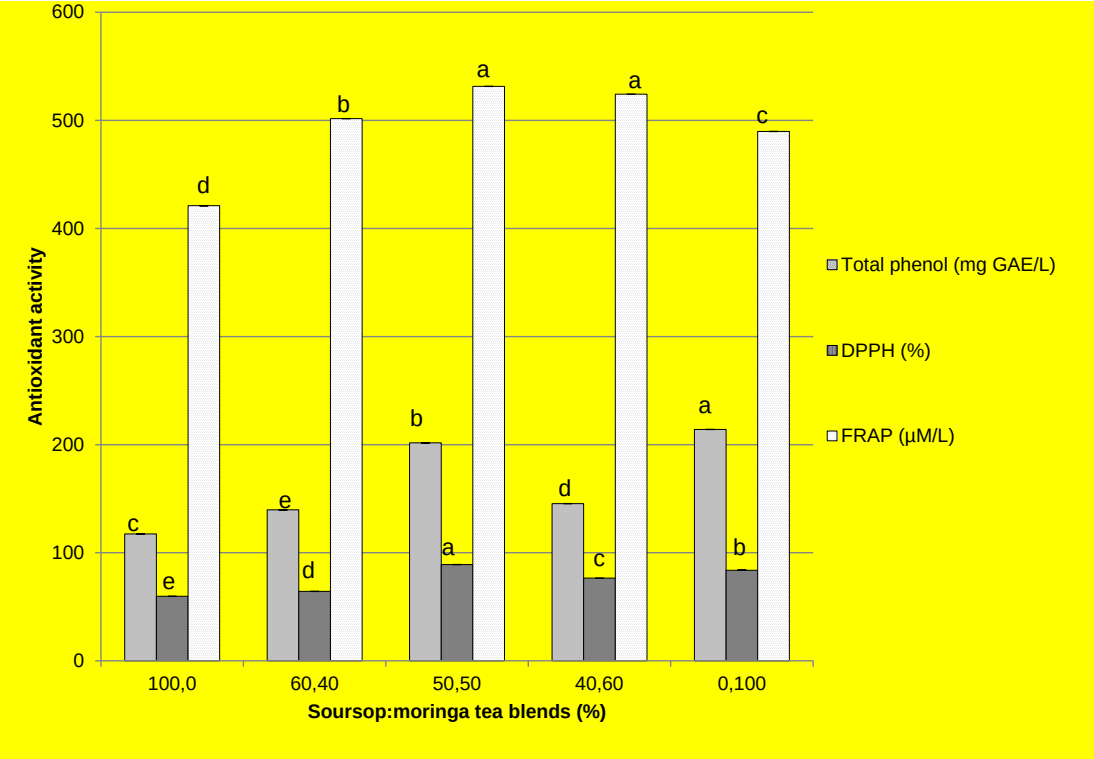

Fig.1: Total phenol and antioxidant activity of soursop-moringa tea extracts
Data are means of triplicate (n=3) analysis ± standard deviation. Means with the same superscripts on similar bars are not significantly different ($p > .05$) and means with different superscript on similar bars are significantly different ($p<.05$). 100% soursop tea (100:0), 60% soursop:40%moringa (60,40), 50% soursop:50%moringa (50:50), 40% soursop:60%moringa (40:60), 100%moringa tea (0:100).

4. CONCLUSION

The chemical composition and antioxidant activity of tea made from blends of dried moringa and soursop leaves were evaluated. Vitamins C and A were analyzed and the highest values obtained for soursop:moringa tea (50:50%) suggest the potential of this tea to supply recommended daily intake for both vitamins and protect the body against free radicals as antioxidants. Seven minerals analyzed in this study showed significant differences ($p<0.05$) among the tea samples. Calcium and potassium were dominant in all the tea blends. Although the 100% moringa tea showed higher levels of the minerals than 100% soursop tea, the highest levels of calcium, sodium, magnesium, phosphorus, zinc, and potassium were observed with the soursop-moringa 50:50%. Basic pH was recorded for all the tea samples. This property makes soursop:moringa tea suitable for individuals with sensitive teeth against the acidic tea. Antinutrients were present in the tea blends with the highest level found in 100% moringa tea. High tannin content observed in the tea blends could benefit people with iron overload reducing the need for blood removal. The values obtained

for all the antinutrients tested were within the acceptable limits. The level of total phenol obtained in this study suggests the potential of the tea products having good antioxidant activity. Antioxidant effect was most evident with 50% inclusion of both soursop and moringa leaves as revealed by the high values of FRAP and DPPH. Overall soursop:moringa tea blend (50:50%) emerged the best in all the quality parameters evaluated and demonstrated the potentials of soursop and moringa leaves in the production of tea.

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