Original Research Article

Evaluation of Phytochemical, Nutritional and Antioxidant Properties of a Functional Tea Produced from Blends of Local Spices Utazi (*Gongronema latifolium),* Nchanwu (*Ocimum gratissium)* and Tumeric (*Curcuma longa).*

ABSTRACT

The study assessed the phytochemical, nutritional and antioxidant properties of a functional tea made from mixture of Utazi leaf *Gongronema latifolium),* Nchanwu leaf (*Ocimum gratissimum)* and Turmeric root (*Curcuma longa).* The three spices were individually processed by drying and mincing before combining them at different ratios that resulted to 16 samples. The samples were generated through D-optimal mixture design using statistical software (Design Expert version 12). The samples were evaluated for phytochemical, nutritional and antioxidant properties. The fresh raw materials were destalked (for the leaves), sorted, washed, withered, oxidized for 24 h, oven dried at 60^{o}C for 30 min, cooled and milled, and packaged. Fresh turmeric root were sorted, washed blanched at 90^{o}C for 2 min, cooled, peeled, sliced, oven dried at 60^{o}C for 30 min, cooled, milled and packaged. The product were mixed at different proportions and packaged in a tea bag and placed in a high density polyethylene. The alkaloids, saponin, phenol, cardiac glycoside, flavonoid, tannin, steroid and anthocyanin content ranged from 12.00-22.00, 0.42-1.15, 11.67-15.63, 0.06-0.67, 12.67-22.00, 0.56-0.89, 1.00-1.67, 46.00-54.00 mg/100g respectively. The vitamin C and E content ranged from 3.03-4.13, 0.31-0.64 mg/100g respectively. The minerals iron and zinc content of the tea samples ranged from 3.53-4.70, 0.06-0.78 ppm respectively. The antioxidant scavenging activity ranged from 42.00-47.00% with sample EC exhibiting the highest level of activity, and the proportion was 70g, 24.62g, 5.38g of Utazi leave, Nchanwu leave, and Tumeric root respectively. Therefore, the functional tea product is a good source of nutrient that is rich in phytochemicals and antioxidants that ensures sound health and aliment management.

Keywords: Functional tea, antioxidant, DPPH, phytochemical, mixture design

1. INTRODUCTION

Tea is a hot drink made by infusing tea leaves in hot water that could be taken with milk or lemon and/or sugar added (Oxford Advanced Learner's Dictionary, [1]. The word 'tea' can also be used to describe an infusion made from herbs, spices and dried fruits of *Camellia sinensis*. Tea is the second most widely consumed beverage globally after water and can be prepared as cold or hot infusions (Dufresne and Farnworth, [2]; Sharma et al. [3]. Tea is one of the most popular and lowest cost beverages in the world and consumed by a large number of people Oluyole et al. [4]. Generally, herbal tea is a functional beverage obtained from plants other than *Camellia sinensis*, by infusing the leaves, stems, barks, seeds or roots, in boiling water. Tea is one of the most common as well as cheapest commodities, which is consumed in more than 65 countries all across the world. It serves as a source of revenue for several tea producing countries and is a very important export commodity. Tea has been reported to have many health benefits including anti-tumour, anti-carcinogenic, anti-arteriosclerotic, antioxidant and many more protective properties Adnan et al. [5]. Being a major export commodity, tea serves to contribute significantly to India's foreign exchange earnings.

Herbal teas, on the other hand look like teas and is brewed the same way but are not considered tea because they do not originate from the *Camellia sinensis* plant. Herbal teas are more precisely known as 'tisanes' connoting they are derived from blends of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanic sources (Kara, [6] Ravikumar, [7]. Teas and herbal teas do not only serve as refreshing drinks but also have some medicinal values. A variety of compounds such as polysaccharides, antioxidants and this is unassociated with the high polyphenol content they possess. In Nigeria, leaves such as Bitter leaf, Utazi leaf and Uziza leaf, have been effectively utilized for the manufacture of herbal teas due to their medicinal properties Okafor et al. [8]. Tea increases alertness and also speeds up heartbeat and breathing rate thus reduces the incidence of hypotension Aroyeun et al. [9]; Oluyole et al. [10].However, most teas circulating in the Nigerian market are imported.

The aim of this work is to produce an acceptable functional tea from a blend of *Gongronema latifolium, Ocimum gratissimum, Curcuma longa* and to evaluate the phytochemical, nutritional composition and antioxidant properties of the tea with the addition of optimizing the response variables to produce the optimal blends of the functional tea.

2.0. MATERIALS AND METHODS

2.1. Source of Materials

Fresh and healthy Utazi leaf *(Gongronema latifolium),* Nchanwu leaf *(Ocimum gratissimum)* and Tumeric root *(Curcuma longa)*, and were purchased from New Market in Enugu State. The analyses were carried out at the Central laboratory of National Abbrovirus Vector Control and Research Institute Enugu State.

The blended tea product were packaged in an infusible tea bag as the primary package material and then in an air tight container high density polyethylene as the secondary packaging material.

The equipment and chemicals used were of analytical grade.

2.2. Preparation of the Sample

2.2.1. Utazi and Nchanwu leaves

The plant leaves were destalked and sorted to remove discoloured and diseased ones, then carefully washed with clean water to remove soil particles after which the leaves were drained of water using a sieve. The leaves were withered (controlled) by spreading them thinly on clean jute bags and conventionally drying for 12 h in a controlled temperature. The dried samples were partially oxidized for 24 h at 25-30ºC. The leaves were cooled to room temperature. The leaves were oven-dried immediately in a hot air oven (Labaids hot air oven; model number 1201; India) for 30 min at 60^{o}C. The dried leaf samples were subjected to blending machine to obtain powdery-coarse products that were packaged in an air tight container which were subjected to further analysis.

2.2.2. Turmeric rhizome

The turmeric rhizomes were sorted to remove damaged ones from the good ones, washed thoroughly with clean water to remove soil particle from them and blanched for 2 min at 90^{o}C to inactivate enzymes and then the roots were peeled and sliced thinly into 0.2 mm sizes to increase surface area. The sliced turmeric rhizomes were oven-dried (Labaids hot air oven; model number 1201; India) at 60°C for 30 min, the dried roots were milled into powdery-coarse product using a blending machine and stored in air tight containers for further analysis.

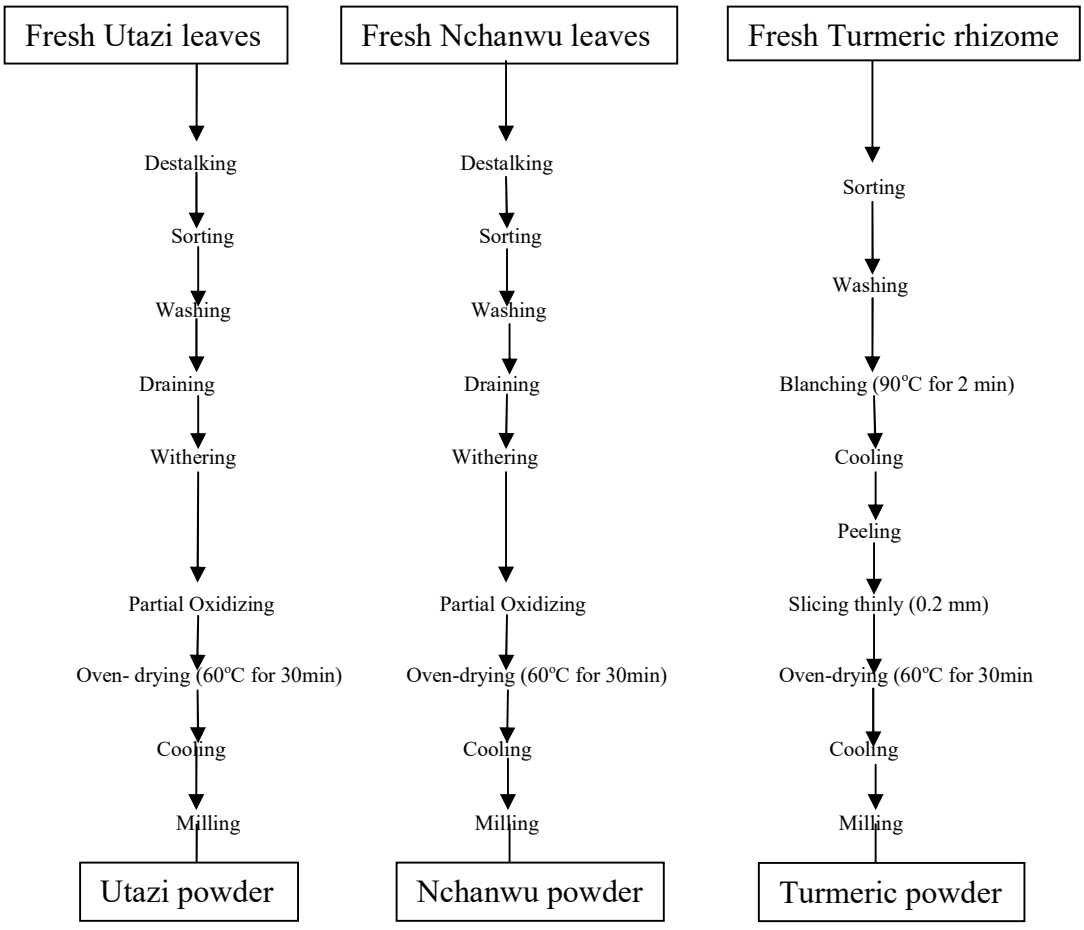

Fig. 1: Processing of the Plant material used in the formulation of functional tea.

2.3. Functional Tea processing steps

2.3.1. Destalking

The leaves of Utazi and Nchanwu were separated from the stems.

2.3.2. Sorting

Discolored and diseased leaves were separated manually (by hand) from the healthy leaves

2.3.3. Washing

The sorted leaves and roots were thoroughly washed in potable water to remove adhering dirt and soil particles.

2.3.4. Withering

Withering refers to the wilting of fresh tea leaves. The purpose of withering is to reduce the moisture content in the leaves and to allow the flavour compounds to develop. The leaves were subjected to a controlled withering which will take place indoors by laying them out thinly on jute bags and conventionally dried. During the course of withering, the moisture content in the leaf goes down by about 30%, making the leaf look limp and soft enough for rolling. The leaves were withered for a period of time (12 h) at low temperature (10°C–15°C). A short wither allows the leaves to retain a greenish appearance and grassy flavours while a longer wither darkens the leaf and intensifies the aromatic compounds.

2.3.5. Partial Oxidizing

In order to bring out specific intensities in flavours, controlled-oxidation were carried out in a large room where the temperature is maintained at 25-30ºC and humidity is maintained at 60-70%. Here, withered and rolled leaves were spread out on jute bags left to ferment for 24 h. Oxidation results in the browning of the leaves and intensification of their flavour compounds. From the moment they are plucked, the cells within the tea leaves are exposed to oxygen and the volatile compounds within them begin to undergo chemical reactions.

2.3.6. Drying

In order to keep the leaves moisture-free, they were oven-dried (Labaids hot air oven; model number 1201; India) at 60^{o}C for 30 min, drying enhances a tea's flavors and ensures its long shelf-life.

2.3.7. Cooling

The dried leaves were cooled to room temperature.

2.3.8. Sorting (Turmeric rhizome)

The turmeric roots were manually sorted to remove spoilt and damaged ones.

2.3.9. Washing

The roots were thoroughly washed with clean water to remove soil particles.

2.3.10. Blanching

The roots were blanched by immersion in water at temperature of 90^{o}C for 2 min.

2.3.11. Cooling

The blanched roots were cooled immediately to room temperature.

2.3.12. Peeling

The outer skin of the roots was removed manually with kitchen knife.

2.3.13. Slicing

The roots were thinly sliced into 0.2 mm size to ease drying.

2.3.14. Oven-drying

The roots were oven-dried at 60^{o}C for 30min, to reduce the moisture content to 5-6% and to reduce microbial activities.

2.3.15. Milling

The dried leaves of Utazi, Nchanwu and Turmeric root were milled separately using a plate milling machine to get a powdery-coarse product. Then using a kitchen scale different portion of the products was blended together.

2.3.16. Packaging

The tea blends were packaged in a tea bag and packaged in an air tight container.

2.4. Experimental Design

The experiment was designed in constraint mixture (D-optimal) using Design Expert Version 12. There were 3 components in each blend that gave total weight of the

mixture as 100 g as in Table 1 Utazi leaf (60-70 g), Nchanwu leaf (20-30 g) and Turmeric (1-10 g). The design produced 16 runs at varied blends of the various components as shown in Table 2.

Table 1: Low and high for mixture components and experimental design

Mixture component	Low	High
Utazi leaf (g)	60	70
Nchanwu (g)	20	30
Tumeric root (g)	1	10

Table 2: Experimental matrix

S/N	Sample code	Utazi leaf (g)	Nchanwu leaf (g)	Turmeric root (g)
1	AC	64.61	30	5.39
2	BC	65.23	25.04	9.73
3	CA	65.23	25.04	9.73
4	CD	70	20	10
5	EC	70	24.62	5.38
6	CF	69.41	29.59	1
7	GC	69.41	29.59	1
8	DA	70	22.36	7.64
9	AB	70	24.62	5.38
10	HA	60	30	10
11	DC	66.36	26.89	6.76
12	EF	66.36	26.89	6.76
13	JA	67.54	28.52	3.94
14	KF	64.61	30	5.39
15	EA	63.22	28.12	8.66
16	GB	67.41	22.59	10
17	LC (Control)			

2.5. Analysis

2.5.1. Preparation of the Extract

Samples of the tea product (50 g) were macerated with 50 mL of ethanol for 72 h at room temperature. Each extract were filtered (Whatman No. 1 filter paper) and the residue re-extracted with the same solvent. The extracts were combined and concentrated in a rotary evaporator under reduced pressure to give the ethanol extract for phytochemical analysis and antioxidant- activity assay AOAC, [11].

2.5.2. Qualitative and Quantitative Phytochemical analysis

Chemical tests were carried out on the ethanol extracts and on the blended local spices to test for alkaloid, saponin, phenol, cardiac glycoside, flavonoid, tannin, and steroid using standard procedures of Sofowora [12].

2.5.3. Test for Alkaloid: Mayers test

5 ml of filtrate, a drop of Mayer's reagent(1.4g of mercury chloride and 1.0g of potassium iodide was dissolved in 100ml of distilled water) was added along the sides of tube. A creamy white precipitate is formed, indicates the presence of alkaloids.

2.5.4. Test for Cardiac glycosides

2 g of samples were mixed with 30ml of distilled water and boiled for 5 minutes in a water bath. The mixture was cooled and filtered. To 5ml of the filtrate, 0.2ml of Fehling's solution A and B were added and boiled further in a water bath for 2 minutes. A brick red coloration which indicates the presence of glycosides was noticed.

2.5.5. Test for Steroid

0.3g of each sample was weighed into a beaker was mixed with 20 cm^3 of ethanol; the component was extracted for 2 hours. To the ethanolic extract of each sample (5 cm^3) was added 2 cm^3 acetic anhydride followed with 2 cm^3 of concentrated tetraoxosulphate (VI) acid. A violet to blue or green colour change in sample(s) indicates the presence of steroids.

2.5.6. Test for flavonoid

To 5ml of dilute ammonia solution, extract was added, following by addition of concentrated sulphuric acid in side of the tube. Appearance of yellow coloration indicates the presence of Flavanoids.

2.5.7. Test for Tannin

5g of dried powder was stirred with 10ml of distilled water. This was filtered and 0.1% ferric chloric reagent was added to the filtrate. A blue black precipitate indicates the presence of tannin

2.5.8. Test for Saponin

The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of stable foam shows the presence of saponins.

2.5.9. Determination of Alkaloid

The alkaloid content was determined gravimetrically. 5 g of each of the samples were weighed using a weighing balance and dispersed into 50 mL of 10% acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 h before it is filtered. The filtrate was then evaporated to one quarter of its original volume on a hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and it was then washed with 1% ammonium hydroxide solution. The filter paper containing the precipitate was dried on an oven at 60^{o}C for 30 min, transferred into

desiccators to cool and then reweighed until a constant weight was obtained. The constant weight was recorded. The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed.

2.5.10. Determination of Tannin

Folin-Denis spectrophotometric method

1.0g of each of the sample was measured and dispersed in 10ml of distilled water before it was agitated. This was allowed to stand for 30 min at room temperature while continuously stirring every 5mins. At the end of 30 min, it was centrifuged and the extract was obtained. 2.5 mL of the extract was dispersed into a 50 mL in volumetric flask. Similarly, 2.5 mL of standard tannic acid was dispersed into a separate 50 mL flask. A 1.0 mL Folin-Denis reagent was measured into each flask followed by the addition of 2.5mL of saturated Na_2CO_3 solution. The mixture was diluted and made up to the 50 mL mark of the flask and was incubated for 90 min at room temperature. The absorbance was measured at 250 nm in a UV spectrophotometer; readings were taken with the blank sample at zero.

The tannin content was given as follows;

$$\% \text{ Tannin} = \frac{An \times C \times 100 \times V_f}{As \times W \times V_A}$$

An= absorbance of the test sample

As = absorbance of standard solution

C = concentration of standard solution

W = weight of sample used

VF= total volume of extract

VA= volume of extract analyzed

2.5.11. Determination of Saponin content

20g of the samples were weighed into a conical flask and added with 200 cm^3 of 20% aqueous ethanol. The sample was heated at 55^oC for 4 hr in water bath with continuous shaking and then filtered off. The residue was rinsed with another 200ml of 20% ethanol and filtered. The filtrate obtained was then concentrated to 40 ml using rotary evaporator (Buchi). The concentrated solution was transferred into a 250 ml separating funnel and added with 20 ml of diethyl ether before the mixture was vigorously shaken. The aqueous layer of solution was recovered while the ether layer was discarded. Afterward, the purification process was repeated twice using 60 ml of n-butanol. The purified sample was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated to dryness using rotary evaporator and dried in oven to a constant weight. The mass value was calculated as a percentage of saponin contain in sample.

2.5.12. Determination of Flavonoid content

Total flavonoid content was determined (using a colorimetric method). The Stock solution was prepared to get a final concentration of 0.04 mg/100ml of a reaction mixture. After that 2 ml of the stock solution was taken and mixed with 2 ml of 2% aluminum trichloride in methanol. After 10 min, the absorbance was measured at 415 nm using a spectrophotometer (Perkin Elmer) against a blank sample containing of 2 mL methanol extract without $AlCl_3$. The total flavonoid content was determined using a standard curve in range concentration of 0.001 – 0.01 mg/ml and expressed as mg of quercetin equivalents (QE)/g of extract. All the experiments were performed thrice and the results obtained were averaged.

10 g of the sample were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whattmann filter paper No 42 (125 mm). The later transferred into a crucible and evaporated into dryness over a water bath and weighted to a constant weight.

2.5.13. Determination of Cardiac Glycosides

The samples were weighed into a 250 cm^3 round bottom flask and about 200 cm^3 of distilled water was added to one gram of each dry wood powder sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm^3 conical flask containing 20 cm^3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm^3), 8 cm^3 of 6 M NH_4OH (ammonium hydroxide), and 2 cm^3 of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M $AgNO_3$ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the end point.

Content of cardiac glycoside in the sample was calculated as:

$$\text{Cardiac glycoside} = \frac{\text{mg}}{100\text{g}}$$

$$\frac{\text{Titre value } (cm^3) \times 1.08 \times \text{exact volume} \times 100}{\text{Aliquot volume } (cm^3) \times \text{sample weight (g)}}$$

2.5.14. Determination of Total Phenolic Content

Total soluble phenolic content in the plant sample extract were determined with Folin-Ciocalteu reagent according to the method described by Wolfe et al. [13] using Gallic acid as a standard with slight modifications. Briefly, 0.5 mL of each extract (methanol and aqueous) were measured into a volumetric flask. About 5 mL of Folin-Ciocalteu reagent were added and the content in the volumetric flask mixed thoroughly. After 3 min, 4 mL of Na_2CO_3 (75 g/L) were added, and allowed to stand for 30 min with intermittent shaking and the absorbance were read against a blank at 760 nm from a spectrophotometer (Spectrumlab 752s UV VIS spectrophotometer).

2.5.15. Determination of Total anthocyanin

Total anthocyanin content was determined using the colorimetric pH differential method. Using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) (125 mL of 0.2mol/L KCl and 375 mL of 0.2 mol/L HCl) and sodium acetate buffer, pH 4.5 (0.4 mol/L HCl) (400 mL of mol/L sodium acetate, 240 mL of 1 mol/L HCl and 360 mL of water). One mL of sample was diluted in 4 mL of each buffer to achieve the absorbance readings at 520nm or at 700nm between 0.2 and 0.4. The diluted samples were allowed to stand for 15 min at the dark room before the absorbance was read at 520nm and 700nm, Spectrumlab 752s UV VIS spectrophotometer, with distilled water as the blank.

Anthocyanin concentration was expressed as cyaniding-3-glucoside equivalent as follows.

$$\text{Anthocyanin (cyanin-3- glucoside mg/L} = \frac{100\text{-A}\times\text{Mw}\times\text{DF}\times1000}{\text{E} \times 1}$$

A is the absorbance of the sample calculated as $(A_{520}\text{-}A_{700})$ pH 1.0× $(A_{520}\text{-}A_{700})$ pH4.5

Mw = molecular weight, Df, dilution factor, E= molar absorptivity

2.6. DPPH assay for Antioxidant Activity

The ability of the extract to scavenge DPPH radical was determined according to Mensor et al. [14] with little modification. 1.0 mL of 0.3 M DPPH methanol solution were added to the solution of the extract or standard (250 ug/mL, 2.5 mL) and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture were measured at 518 nm with spectrophotometer and converted to percentage antioxidant activity (AA%). Methanol (1.0 mL plus extract solution (2.5 mL) were used as a blank 1.0 mL of 0.3 M DPPH plus methanol (2.5 mL) was used as a negative control. Solution of ascorbic acid served as positive control. Antioxidant activity (AA) was calculated as percentage inhibition relative to control using the following equation.

DPPH scavenging effect (%) =

$$\frac{Ao - A1\ X\ 100}{Ao} \quad \textbf{.....} \quad \textbf{1}$$

A_1= Absorbance of the reaction mixture/standard. Ascorbic acid was used as control.

A_o= Absorbance of the control (containing all the reagents except the extract).

2.7. Determination of Vitamins

2.7.1. Determination of Vitamin C (Ascorbic acid) Concentration

This was done using a modified method of AOAC [15], where 1 g of each sample was macerated with 5 mL of 0.4% oxalic acid for 10 min. The supernatant (1 mL) will then be transferred into the test tubes to which 9 mL of 2, 6- dichlorophenol in iophenols (12 mg/L) and then mixed thoroughly by shaking. The absorbance of the resulting solution was taken at 520 nm at 15 sec and 30 sec against corresponding blank.

Concentration of Vitamin C (mg) =

$$\frac{Ab \times DF \times pathlength}{E} \quad \textbf{.....} \quad \textbf{2}$$

2.7.2. Determination of Vitamin E (Tocopherol) concentration

This was done using a modified method of AOAC [15], where 1 g of each sample was macerated with 20 mL of petroleum ether for 10 min and allowed to stand for 1 h with intermitted shaking at every 1 min and supernatant (3 mL) was transferred into double test tubes, evaporated to dryness and the residue re-dissolved with 2 mL ethanol and shaken. 1 mL of 0.2% ferric chloride in ethanol and 1 mL of 0.5% a - dipyridyl in ethanol then added to the resulting solution and then made up to 5 mL with ethanol. The mixture was vigorously shook and the absorbance taken at a wavelength of 520 nm against corresponding blank.

Concentration of Vitamin E (mg) =

$$\frac{Ab \times DF \times pathlength}{E} \textbf{.....} \quad \textbf{3}$$

2.8. Determination of Minerals

The mineral concentrations of zinc (Zn), iron (Fe) was determined using the atomic absorption spectrophotometer.

2.9. Statistical analysis

All analysis was performed in triplicate and the results were expressed as Mean $\pm$ Standard deviation. Statistical significance of differences between the samples were determined using analysis of variance (ANOVA), followed by a Duncan test at 95% confidence level using IBM SPSS version 23. Design-Expert version 12 was used to generate the experimental design and mathematical models as well as for construction of the graphs.

The results of the experimental design were exploited in the form of specific response surface for each parameter, in order to determine the optimal blend of tea sample from Utazi leaf, Nchanwu leaf, Turmeric root.

A  B  C 

plate 1

plate A = (*Gongronema latifolium)* Utazi leaves

plate B = *(Ocimum gratissimum)* Nchanwu leaves

plate C = (*Curcuma longa)* Tumeric roots

A  B  C 

plate 2 :

Plate A = (*Gongronema latifolium*) Destalked Utazi leaves.

Plate B = (*Ocimum gratissium*) Destalked Nchanwu leaves.

Plate C = (*Curcuma longa*) Sorted Turmeric roots.

A  B  C 

plate 3 : Oven-dried Plant materials

Plate A = (*Gongronema latifolium*) Utazi leaves.

Plate B = (*Ocimum gratissium*) Nchanwu leaves.

Plate C = (*Curcuma longa*) Turmeric roots.

A  B  C 

plate 4 : Grinded Plant materials

Plate A = (*Gongronema latifolium*) Utazi leaves.

Plate B = (*Ocimum gratissium*) Nchanwu leaves.

Plate C = (*Curcuma longa*) Turmeric roots.

(A)  (B) 

plate 5 : Pictures of Packaged Functional tea blends in (A) and the tea bags in a plate (B)

3. RESULTS

The results of the study were shown in tables 3 to 4

4. DISCUSSION

This research is geared towards the evaluation of phytochemical and nutritional composition, antioxidant of functional tea from blends of *Gongronema latifolium, Ocimum gratissimum and Curcuma longa.*

Table 3: Qualitative analysis of Phytochemicals in the functional tea blends from Utazi leaf (*Gongronema latifolium)*, Nchanwu (*Ocimum gratissimum)* and Turmeric root (*Curcuma longa)*.

S/N	Sample Codes	Alkaloid	Saponin	Phenol	C. Glycoside	Flavonoid	Tannin	Steroid	Quinone
1	AC	+	+	-	+	+	-	-	-
2	BC	-	-	-	+	+	-	-	+
3	CD	-	++	-	-	-	-	-	+
4	EA	+	++	+++	-	-	-	-	+
5	EC	-	+	-	+	-	-	-	-
6	GB	-	+	+	+	-	-	-	-
7	KF	+	++	++	+	-	-	-	-
8	HA	+	+	++	-	+	-	-	+
9	CF	-	+	-	+	+	-	-	-
10	DC	++	++	-	+	+	+	-	-
11	DA	-	+	-	-	+	-	-	+
12	JA	-	-	-	+	-	-	-	-
13	AB	+	+	+	+	+	-	-	+
14	CA	-	-	-	+	+	-	-	+
15	GC	-	+	-	+	+	-	-	-
16	EF	++	++	-	+	+	+	-	-
17	LC (control)	+	+	+++	++	-	+++	-	-

+++ = Abundant, ++ = Moderate, + = Present, - = Absent.

Table 4.: Phytochemical composition (mg/100g) of the functional tea blends from Utazi leaf (*Gongronema latifolium)*, Nchanwu (*Ocimum gratissimum)* and Turmeric root (*Curcuma longa)*.

S/N	Sample Codes	Alkaloids	Saponins	Phenols	C. Glycosides	Flavonoid	Tannin	Steroid	Anthocyanin
1	AC	$13.67^{abcd} \pm 2.08$	$0.73^{bcd} \pm 0.05$	$13.67^{a} \pm 2.08$	$0.28^{ab} \pm 0.11$	$15.67^{a} \pm 2.08$	$0.77^{a} \pm 0.05$	$1.33^{abc} \pm 0.15$	$45.33^{a} \pm 1.53$
2	BC	$14.67^{abcde} \pm 0.58$	$0.97^{bde} \pm 0.03$	$13.04^{a} \pm 2.00$	$0.34^{abc} \pm 0.20$	$15.67^{a} \pm 2.08$	$0.56^{a} \pm 0.22$	$1.27^{ab} \pm 0.12$	$49.33^{d} \pm 2.52$
3	CD	$16.00^{bcde} \pm 3.00$	$0.60^{abc} \pm 0.13$	$12.50^{a} \pm 2.18$	$0.38^{abc} \pm 0.20$	$13.33^{a} \pm 3.21$	$0.61^{a} \pm 0.19$	$1.33^{abc} \pm 0.15$	$52.33^{de} \pm 0.58$
4	EA	$16.67^{cde} \pm 1.53$	$0.74^{bcd} \pm 0.27$	$12.59^{a} \pm 1.03$	$0.48^{bc} \pm 0.23$	$13.67^{a} \pm 4.73$	$0.62^{a} \pm 0.06$	$1.47^{bc} \pm 0.32$	$44.33^{a} \pm 1.15$
5	EC	$17.33^{de} \pm 2.52$	$0.42^{a} \pm 0.16$	$12.26^{a} \pm 1.10$	$0.66^{c} \pm 0.33$	$15.33^{a} \pm 3.06$	$0.60^{a} \pm 0.37$	$1.27^{ab} \pm 0.25$	$49.33^{cd} \pm 2.31$
6	GB	$18.00^{e} \pm 1.00$	$0.52^{ab} \pm 0.17$	$13.70^{a} \pm 1.47$	$0.67^{c} \pm 0.32$	$15.33^{a} \pm 1.53$	$0.71^{a} \pm 0.25$	$1.37^{bc} \pm 0.06$	$54.00^{e} \pm 1.00$
7	KF	$13.00^{abc} \pm 1.00$	$0.44^{a} \pm 0.19$	$13.93^{a} \pm 2.69$	$0.45^{bc} \pm 0.09$	$14.00^{a} \pm 2.65$	$0.77^{a} \pm 0.19$	$1.50^{bc} \pm 0.10$	$50.00^{cd} \pm 1.00$
8	HA	$12.00^{a} \pm 2.00$	$0.43^{a} \pm 0.14$	$14.57^{a} \pm 2.98$	$0.38^{abc} \pm 0.03$	$14.00^{a} \pm 2.65$	$0.89^{a} \pm 0.06$	$1.67^{c} \pm 0.23$	$53.33^{e} \pm 1.53$
9	CF	$17.33^{d} \pm 2.52$	$0.75^{bcd} \pm 0.03$	$15.63^{a} \pm 3.10$	$0.36^{abc} \pm 0.06$	$16.00^{a} \pm 1.00$	$0.83^{a} \pm 0.09$	$1.50^{bc} \pm 0.20$	$54.00^{e} \pm 1.00$
10	DC	$14.33^{abcde} \pm 2.08$	$0.63^{abc} \pm 0.13$	$14.29^{a} \pm 1.58$	$0.45^{bc} \pm 0.14$	$15.33^{a} \pm 2.08$	$0.72^{a} \pm 0.26$	$1.40^{bc} \pm 0.10$	$48.33^{bc} \pm 1.53$
11	DA	$14.33^{abcde} \pm 2.52$	$0.80^{cd} \pm 0.02$	$14.59^{a} \pm 2.17$	$0.25^{ab} \pm 0.12$	$14.00^{a} \pm 3.46$	$0.68^{a} \pm 0.25$	$1.37^{c} \pm 0.25$	$50.00^{cd} \pm 2.00$
12	JA	$12.67^{ab} \pm 2.08$	$0.84^{cd} \pm 0.02$	$13.90^{a} \pm 2.71$	$0.26^{ab} \pm 0.13$	$12.67^{a} \pm 2.89$	$0.70^{a} \pm 0.09$	$1.33^{abc} \pm 0.21$	$47.33^{ab} \pm 2.52$
13	AB	$17.67^{e} \pm 2.08$	$0.82^{cd} \pm 0.08$	$11.67^{a} \pm 0.58$	$0.38^{abc} \pm 0.15$	$13.67^{a} \pm 2.89$	$0.78^{a} \pm 0.12$	$1.23^{ab} \pm 0.21$	$49.67^{c} \pm 2.08$
14	CA	$14.67^{abcd} \pm 0.58$	$0.97^{de} \pm 0.03$	$13.04^{a} \pm 2.00$	$0.34^{abc} \pm 0.20$	$15.67^{a} \pm 2.08$	$0.56^{a} \pm 0.22$	$1.27^{ab} \pm 0.12$	$49.33^{cd} \pm 2.52$
15	GC	$17.33^{de} \pm 2.52$	$0.75^{bcd} \pm 0.03$	$15.63^{a} \pm 3.10$	$0.36^{abc} \pm 0.06$	$16.00^{a} \pm 1.00$	$0.83^{a} \pm 0.09$	$1.50^{bc} \pm 0.20$	$54.00^{e} \pm 1.00$
16	EF	$14.33^{abcde} \pm 2.08$	$0.63^{abc} \pm 0.13$	$14.29^{a} \pm 1.58$	$0.45^{bc} \pm 0.14$	$15.33^{a} \pm 2.08$	$0.72^{a} \pm 0.26$	$1.40^{bc} \pm 0.10$	$48.33^{bc} \pm 1.53$
17	LC(con.)	$22.67^{f} \pm 2.08$	$1.15^{e} \pm 0.19$	$12.33^{a} \pm 2.08$	$0.06^{a} \pm 0.03$	$22.00^{b} \pm 1.00$	$0.57^{a} \pm 0.21$	$1.00^{a} \pm 0.00$	$46.00^{ab} \pm 1.00$

Values are means of triplicate determinations ± Standard Deviation. Values in the same column bearing different superscript differ significantly ($P < 0.05$). LC = Commercial sample.

Phytochemical screening

The result in Table 4.0 shows the presence of the alkaloid, saponin, phenol, cardiac glycoside, flavonoid, tannin, steroid and quinone in varying proportion, but the absence steroid in the tea samples. This entails that the tea samples is a good source of phytochemicals and suggests the presence of medicinal properties in the tea samples.

The result of the phytochemical composition of ethanol extracts of the tea samples revealed that they contain alkaloid, saponin, phenol, cardiac glycoside, flavonoid, tannin, and steroid in varying compositions.

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The ideal polynomial equations are

Linear: $\hat{y}=\sum qi=1\beta ixi$ …….. 1

Quadratic: $\hat{y}=\sum qi=1\beta ixi+\sum q-1i<j\sum qj\beta ijxixj$

…….. 2

Special cubic: $\hat{y}=\sum qi=1\beta ixi+\sum q-1i<j\sum qj\beta ijx$

$ixj+\sum q-2i<j\sum q-1j<k\sum qk\beta ijkxixjxk$……

……3

The parameter βi represents the expected response to the pure blend $x_i = 1$ and $x_j = 0$ when $j \neq i$. The term $\sum qi=1\beta ixi$ represents the linear blending portion. When curvature arises from nonlinear blending between component pairs, the parameters β_{ij}, which represent either synergistic or antagonistic blending, will be different from zero.

Alkaloid

Alkaloids content ranged from 12.00 to 22.00 mg/100g, with the sample HA having the lowest value (12.00 mg/100g) and sample LC having the highest value (22.00 mg/100g), most of the samples differed significantly ($p> 0.05$). This finding was not in accordance to the work of Ndife et al. [16] on Utazi and Nchanwu leaves which was 4.90% and 7.91%. Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants. Alkaloids make up 20% of the known secondary metabolites found in plants, some alkaloids exert a stimulating role on the central nervous system (Kaur and Aurora, [17]. Alkaloids content in plant leaves are known to possess antimalarial, pharmacological, anti-hypertensive, anti-arrhythmic and anticancer effect (Mamta et al. [18].

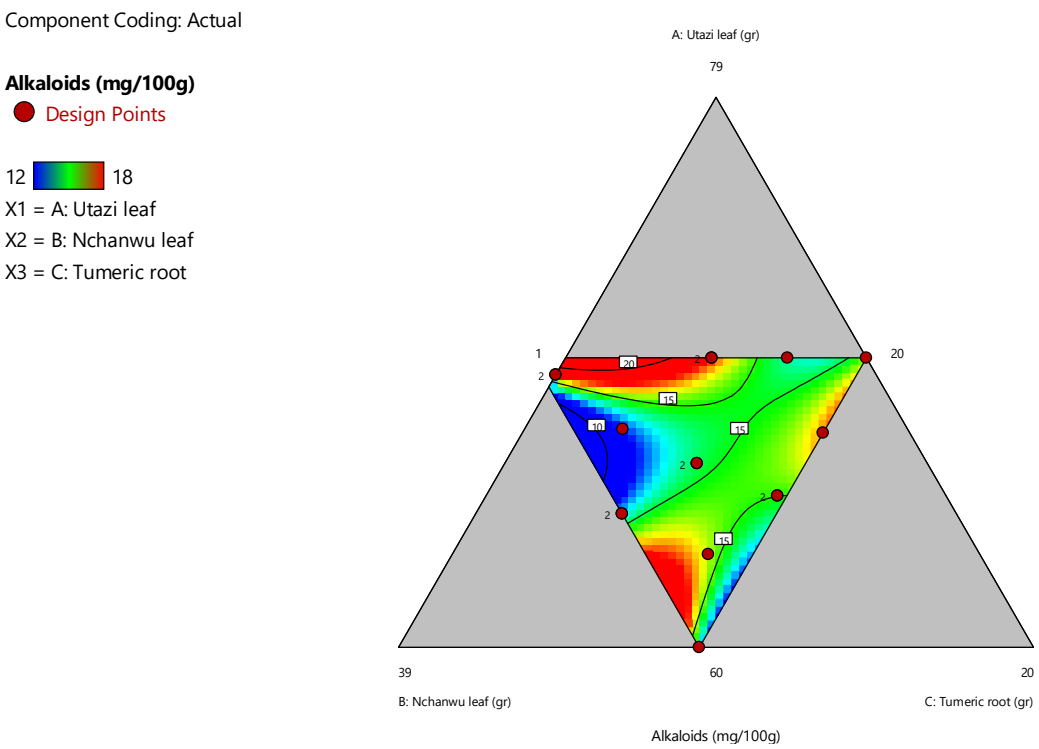

Figure 2**:** Contour Plot of Alkaloid content of Functional tea blends

Alkaloid = A + B + C + AC + BC + AB(A-B) + AC(A-C) + BC(B-C)

Alkaloid = 129.44A + 0.57B – 92.63C – 186.95AC + 212.61BC + 140.81AB(A-B)

–626.09AC(A-C)+205.15BC(B-C)…….. Eq. 4

Alkaloid

Alkaloid content was modeled as shown in Eq. 4 Special Cubic model was suggested by Mixture Design (D-optimal) ANOVA for alkaloid. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. From the ANOVA, only **A**, **B, C, AC, BC, AB(A-B), AC(A-C),** and **BC(B-C)** had p-value < 0.05. Figure 2 shows the contour plot of Alkaloid content, and from the equation above the alkaloid content of the functional tea blends, will decrease if there is an increase in the Turmeric component of the tea blends, however the alkaloid with increase in both Utazi and Nchanwu leaf.

Saponin

Saponin content varied from 0.42 to 1.15 mg/100g, sample EC having the lowest value (0.42mg/100g) and sample LC having the highest (1.15 mg/100g). The samples had significant difference, though some samples were similar. This is consistent with the works of Nduche et al. [19] on Utazi leaf which recorded 0.52mg/100g also in the finding of Ikpeama et al. [20] on turmeric root 0.45mg/100g. Igile et al. [21] reported that saponins at low levels less than 10% are said to be safe and non - toxic, this implies that the functional tea samples are safe for human consumption since they had low saponin content. The low saponin content can associated with the processing steps during production. Igile et al. [21] reported that high saponin levels have been associated with

gastroenteritsis, manifested by diarrhea and dysentery. However saponin has been shown to possess beneficial component such as lowering cholesterol absorption and perhaps glucose, by binding to the cholesterol and bile acid in the intestinal tracts Fila et al. [22].

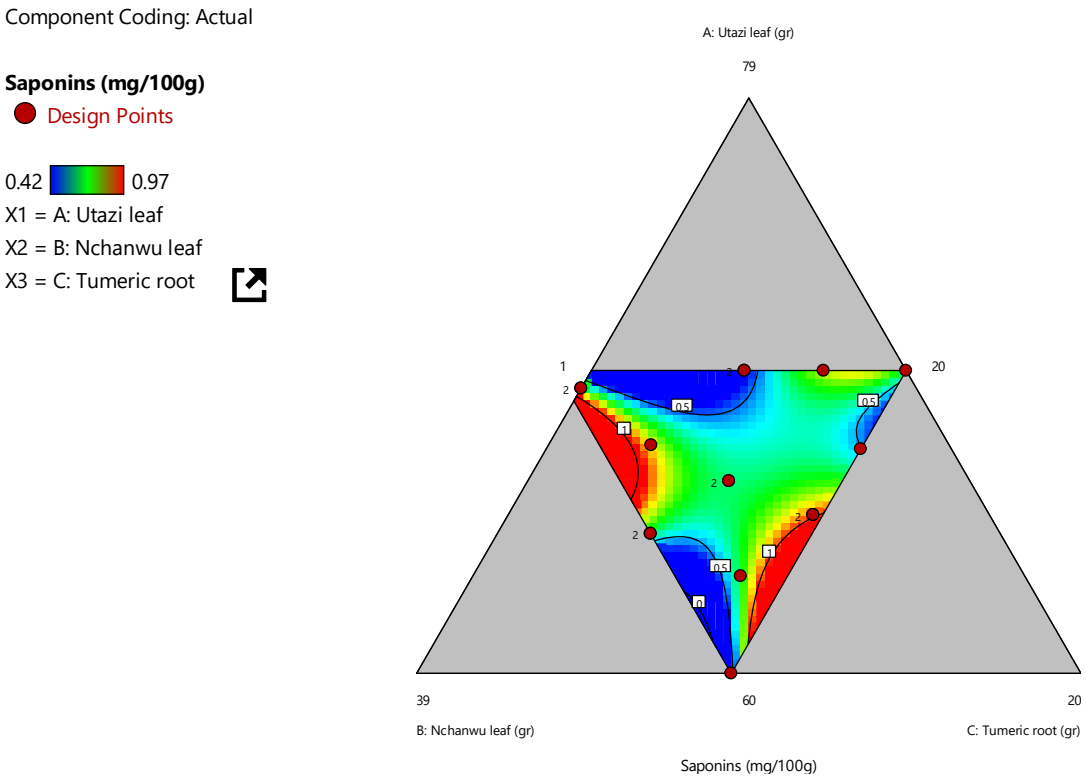

Figure 3**:** Contour Plot of Saponin content of the Functional tea blends

Saponin = A+B+C+BC+AB(A-B)+AC(A-C)+BC(B-C)

Saponin =-1.03A–0.44B+13.69C–21.95BC–35.33AB(A-B)+50.13AC(A-C)-26.53BC(B-C)…Eq. 5

Saponin content was modeled as shown in Eq. 3 Special Cubic model was suggested by Mixture design (D-optimal) ANOVA for saponin content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 3 shows the contour plot of saponin content and from the equation above, the saponin content will increase with increase in the amount of Turmeric root component in the tea blends.

Phenol

Phenol content in samples ranged from 11.67 to 15.63 mg/100g, sample AB having (11.67mg/100g) and samples CF and GC having (15.63mg/100g). There was no significant difference in the results. This is consistent with the findings of Omogbai et al. [23] which recorded that the phenol content on utazi leaf was 15.74 mg/100g. Phenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plant. They have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated disease such as cancer. The inverse relationship between fruit and vegetables

intake and the risk of oxidative stress associated disease, such as cancer, osteoporosis has been partially ascribed to phenolics Scalbert et al. [24].

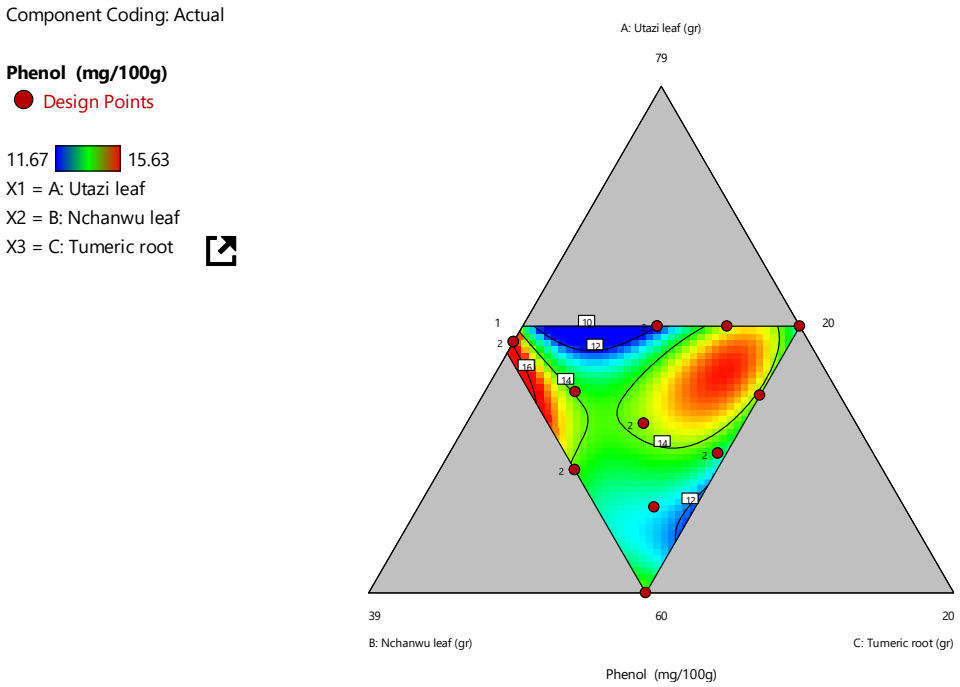

Figure 4: Contour Plot of Phenol content in the Functional tea blends.

Phenol=A+B+C+AB+AC+BC+ABC+AB(A-B)+BC(B-C)

Phenol=-80.64A+54.05B-89.93C+114.27AB+387.31AC+128.42BC-449.88ABC+109.67AB(A-B)-251.62BC(B-C)…….. Eq. 6

Phenol content was modeled as shown in Eq. 6 Special Cubic model was suggested by Mixture design (D-optimal) ANOVA for Phenol content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 4. shows the contour plot of the phenol content and from the equation above increase in the quantity of Nchanwu leaf in the blend will bring about increase in the phenol content.

Cardiac Glycoside

Cardiac Glycoside content in the tea samples ranged from 0.06 to 0.67 mg/100g with sample LC having the lowest value (0.06 mg/100g) and sample GB having the highest value (0.67 mg/100g), the samples differed significantly. Cardiac Glycosides are useful for the treatment of heart conditions.

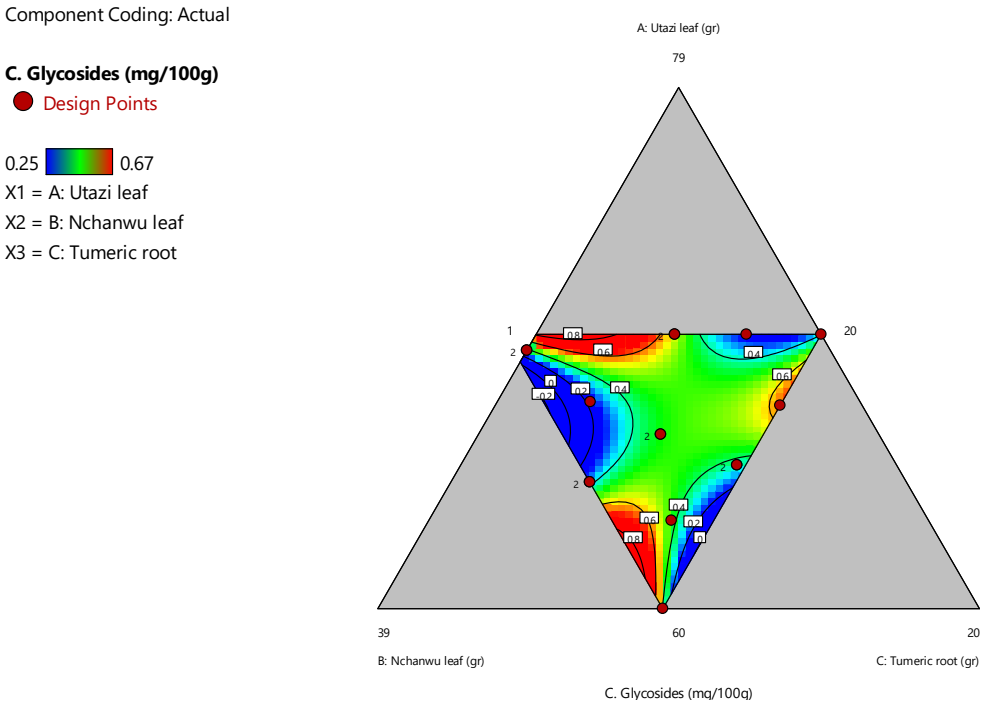

Figure 5: Contour Plot of Cardiac Glycoside of the Functional tea blends.

Cardiac Glycoside = AB(A-B)+ AC(A-C)

Cardiac Glycoside = 23.56AB(A-B) – 43.24AC(A-C)…….. Eq. 7

Cardiac glycoside was modeled as shown in Eq. 7 Special Cubic model was suggested by Mixture design (D-optimal) ANOVA for Cardiac glycoside content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 5 shows the contour plot of the Cardiac glycoside content and from the equation above increase in amount of Utazi leaf and Tumeric root in the blend will lead to in the increase in Cardiac Glycoside.

Flavonoid

Flavonoid content ranged from 12.67 to 22.00 mg/100g, with sample JA having (12.67mg/100g) and sample LC (22.00mg/100g). The only sample with a significant difference was sample LC the commercial sample other blended tea samples were similar. These was not consistent with the work of Nduche et al. [19] on Utazi leaf which recorded 0.37% and the work of Ndife et al. [16] on Nchanwu leaf 4.32%. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer and anti-ulcer activity and protection against the different levels of carcinogenesis Agbaire, [25]; Igile et al. [21].

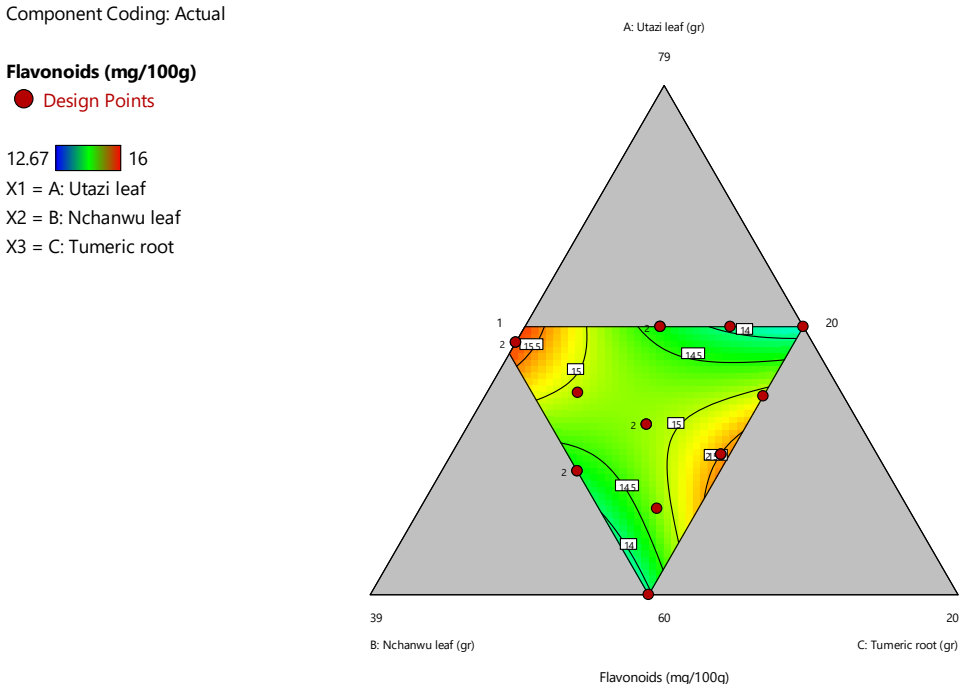

Figure 6**:** Contour Plot of Flavonoid in the Functional tea blends.

Flavonoid = A+B+C+AB

Flavonoid = 9.63A+8.78B+23.65C+26.18AB …….. Eq. 8

Flavonoid was modeled as shown in Eq. 8 Quadratic model was suggested by Mixture design (D-optimal) ANOVA for Flavonoid content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 6 shows the contour plot of the Flavonoid content, from the equation above increase in the Utazi leaf and Nchanwu leaf, component will lead to increase in the flavonoid content of the tea blends.

Tannin content ranged from 0.56 to 0.89 mg/100g, with sample CA and BC having the lowest value (0.56 mg/100g) and sample HA having the highest value (0.89 mg/100g). There was no significant difference observed in the samples. This is consistent with the findings of Ikpeama et al. [20] on turmeric the tannin content recorded 1.08 mg/100g. Tannins are basically used for the treatment of imflammation leucorrhoea, gonorrhea, piles, intestinal disorder such as diarrhea and dysentery Dharmananta, [25], Buzzini et al. [26]. Tannin exerts antimicrobial activities by iron deprivation, hydrogen bonding or interaction with vital proteins such as enzymes in microbial cells Scalbert, [27].

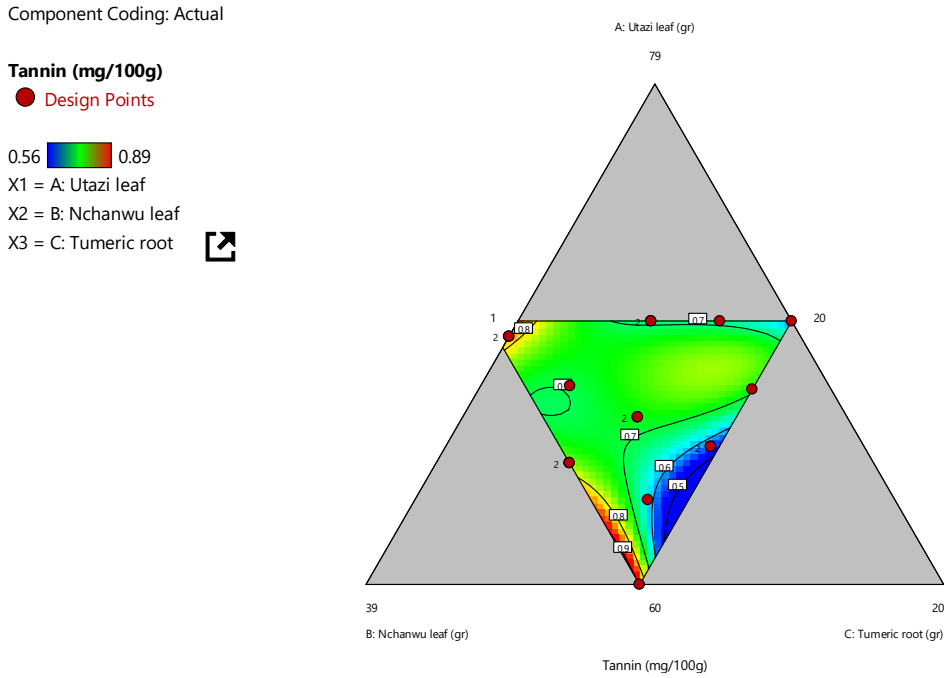

Figure 7: Contour Plot of the Tannin content of the Functional tea blends.

Tannin = A+B+C+AC+AB(A-B)

Tannin = -3.38A+3.78B-6.38C+22.11AC+15.85AB(A-B) …… Eq. 9

Tannin was modeled as shown in Eq. 9 Special Cubic model was suggested by Mixture design (D-optimal) ANOVA for tannin content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 7 shows the contour plot of the tannin and from the equation above increase in the component of Utazi leaf and and Tumeric root will lead to decrease in the tannin content in the tea blends.

Steroid content of the tea blend ranged from 1.00 to 1.67 mg /100g, with sample LC having the lowest value (1.00 mg/100g) and sample HA having the highest value (1.67 mg/100g). The values of the samples had significant difference; the commercial sample LC had slightly lower value than other samples making the tea blends good source of steroid. This is not consistent with work of Ikpeama et al. [20] on turmeric the result showed 0.03% of steroid. Steroid in plants also have many interesting medicinal and pharmaceutical activities such as anti-tumor, immunosuppressive, hepatoprotective, and antibacterial. They have been related with function that boosts sex hormone Okwu and Josiah [28].

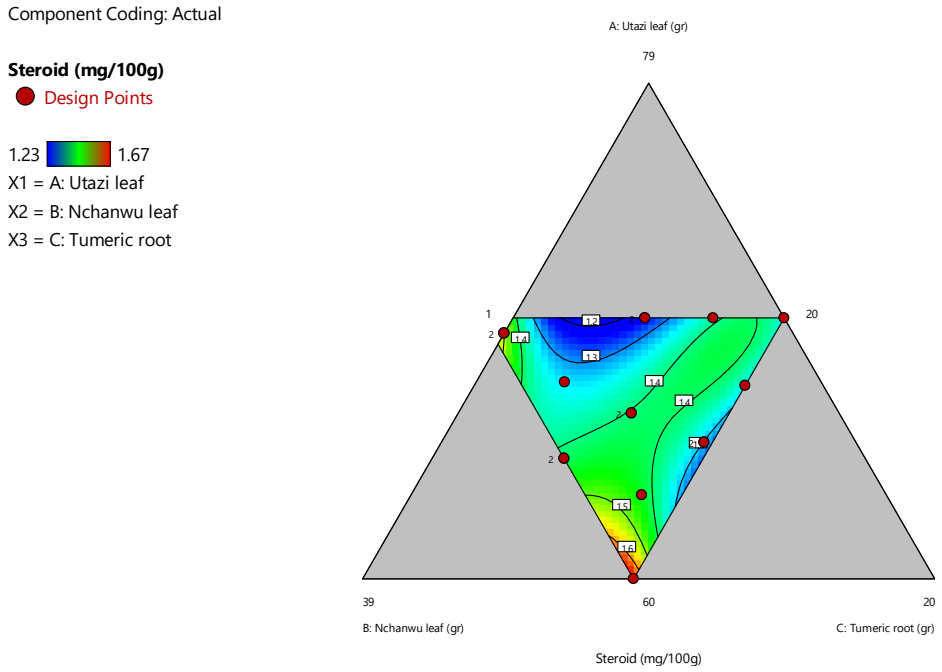

Figure 8**:** Contour Plot of Steroid content in the Functional tea blends.

Steroid =A+B+C+AB+AC+BC+A^{2}BC+AB

Steroid = -2.09A+0.11B-4.51C+9.92AB+18.35AC+15.06BC-31.81A^{2}BC-59.75AB……Eq. 10

Steroid was modeled as shown in Eq. 10. Special Quartic model was suggested by Mixture design (D-optimal) ANOVA for steroid content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 8 shows the contour plot of Steroid and from the equation, increase in the amount Utazi and Nchanwu leaf will bring about increase in the steroid content of the tea blends.

Anthocyanin content ranged from 46.00 to 54.00 mg/100g with sample LC having the lowest value (46.00 mg/100g) and sample GB, CF and GC having the highest value (54.00 mg/100g). This result was similar to the findings of Arnnok et al. [29] on chilli pepper which recorded 62.9 mg/100g, however it was not consistent with the work of Ndife et al. [19] with the findings on Utazi and Nchanwu leaf 0.00% and 0.03% respectively. Anthocyanins are class of natural flavonoids present in plant and are responsible for colouring pigment in leaves, fruits, and flowers. They also have antimicrobial properties, it boosts immune system and fights against inflammation, cancer, heart disease, and it can protect eye cells from damage.

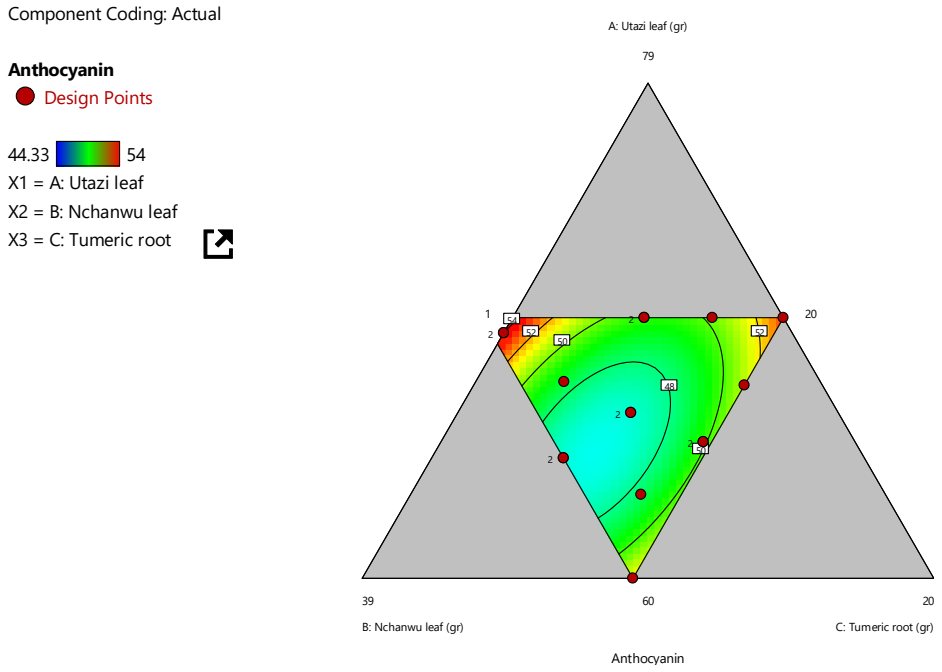

Figure 9: Contour plot of Anthocyanin content of the Functional tea blends.

Anthocyanin = A+B+C+AC+BC

Anthocyanin = 69.89A+56.66B+89.89C-103.43AC-81.94BC…… Eq. 11.

Anthocyanin was modeled as shown in Eq. 11. Quadratic model suggested by Mixture Design D-optimal ANOVA for Anthocyanin. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 9. shows the contour plot of Anthocyanin and from the equation increase in the Turmeric root component will lead to increase in the anthocyanin content of the tea blends.

The values of the antinutrient; tannin, saponin and glycoside in functional tea blends were recorded to be low these was brought about by the processing steps in the production making it is safe to consumption. The high phytochemical composition of the functional tea blends could be as a result of the blending of the plant materials Utazi leaves, Nchanwu leaves and Tumeric root to yield strong therapeutic and medicinal properties.

Table 5: Mineral composition (ppm) of functional tea blends from Utazi leaf (*Gongronema latifolium)*, Nchanwu (*Ocimum gratissimum)* and Turmeric root (*Curcuma longa)*.

S/N	Sample Codes	Zinc Content	Iron Content
1	AC	$0.78^{d} \pm 0.22$	$4.13^{a} \pm 0.78$
2	BC	$0.60^{bcd} \pm 0.06$	$4.23^{a} \pm 0.59$
3	CD	$0.53^{bcd} \pm 0.12$	$4.40^{a} \pm 0.66$
4	EA	$0.56^{bcd} \pm 0.16$	$4.63^{a} \pm 0.86$
5	EC	$0.37^{b} \pm 0.11$	$4.40^{a} \pm 1.15$
6	GB	$0.66^{cd} \pm 0.13$	$4.70^{a} \pm 1.00$
7	KF	$0.67^{cd} \pm 0.05$	$4.37^{a} \pm 1.33$
8	HA	$0.67^{cd} \pm 0.18$	$3.57^{a} \pm 0.51$
9	CF	$0.41^{bc} \pm 0.14$	$3.67^{a} \pm 0.38$
10	DC	$0.58^{bcd} \pm 0.08$	$4.20^{a} \pm 0.69$
11	DA	$0.55^{bcd} \pm 0.19$	$4.13^{a} \pm 0.74$
12	JA	$0.37^{b} \pm 0.14$	$4.60^{a} \pm 0.20$
13	AB	$0.51^{bc} \pm 0.23$	$4.10^{a} \pm 0.36$
14	CA	$0.60^{bcd} \pm 0.06$	$4.23^{a} \pm 0.59$
15	GC	$0.41^{b} \pm 0.14$	$3.67^{a} \pm 0.38$
16	EF	$0.58^{bcd} \pm 0.08$	$4.20^{a} \pm 0.69$
17	LC(control)	$0.06^{a} \pm 0.01$	$3.53^{a} \pm 0.35$

Values are means of triplicate determinations ± Standard Deviation. Values in the same column bearing different superscript differ significantly ($P < 0.05$).

Zinc and iron

Minerals act as catalyst for enzymes during normal metabolic processes, and are considered to be nutritionally and clinically recommended. The zinc content ranged from 0.06 to 0.78 ppm with sample LC having the lowest values (0.06 ppm) and sample AC having the highest value (0.78 ppm). There were significant differences among the blended tea samples. These findings were relatively similar with Enemor et al. [30] work on Utazi were the value of zinc recorded (0.91 ppm). The zinc content is essential in the diet as it ensures a sound immune system, metabolism, and DNA synthesis.

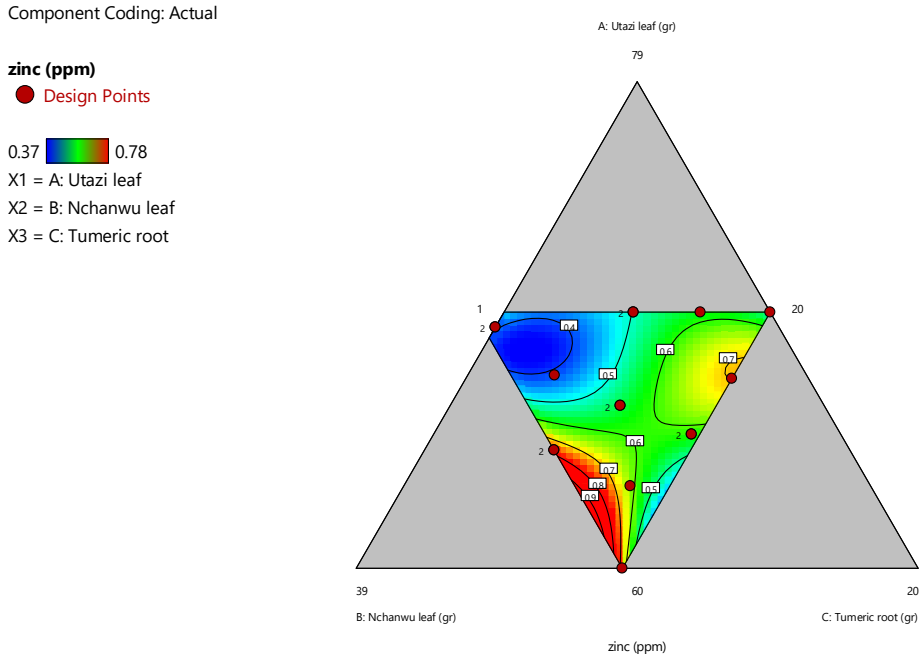

Figure 10**:** Contour plot of Zinc content in the functional tea blends.

Zinc =A+B+C+AB+AB(A-B)-AC(A-C)

Zinc =1.26A+7.00B-5.38C-14.89AB+13.38AB(A-B)-17.02AC(A-C)…….. Eq. 17

Zinc content was modeled as shown in Eq. 17. Special Cubic model suggested by Mixture Design D-optimal ANOVA for zinc content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 10 shows the contour plot of zinc and from the equation increase in the amount of Turmeric root component will bring about decrease in the zinc content of the tea blends.

Iron content ranged from 3.53 to 4.70 ppm, with sample LC having the lowest value (3.53 ppm) and sample GB having the highest value (4.70 ppm). There were no significant differences in the tea samples. These results were not consistent with the work of Enemor et al. [30] but there were close to the range, the iron content in utazi leaf was (5.90 ppm) this could be as a result of processing of the plant leaves into tea. Iron is essential for prevention of anaemia. The iron content present in the functional tea can help in heamoglobin formation Latunde - dada, [31] and hence recommend for iron deficiency anaemia.

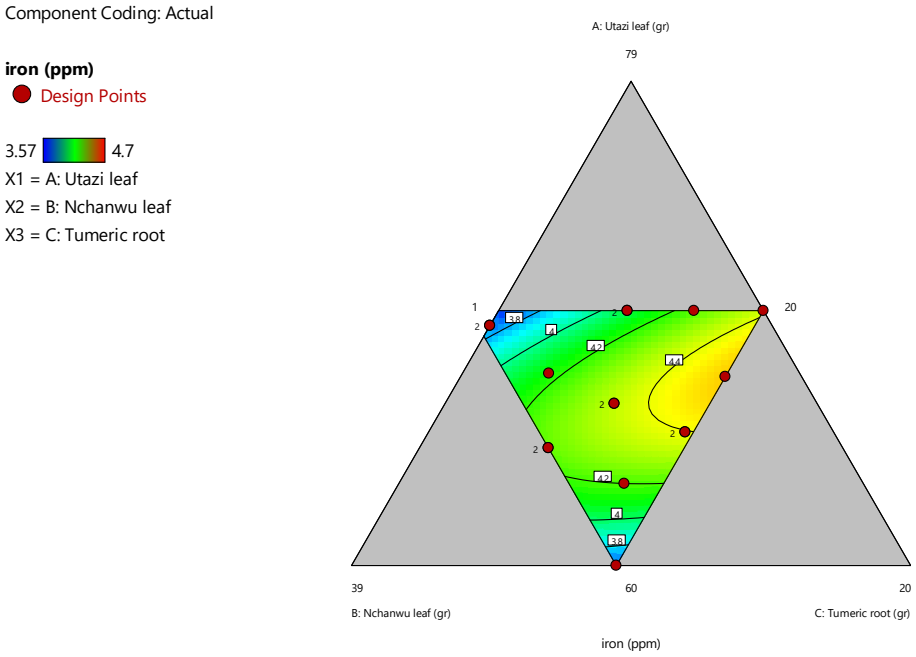

Figure 11**:** Contour plot of Iron content in the functional tea blends.

Iron =A+B+C+AC

Iron=1.09A+3.60B+2.94C+9.68AC……
……..Eq. 18.

Iron content was modeled as shown in Eq. 18. Quadratic model suggested by Mixture Design D-optimal ANOVA for iron content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 11 shows the contour plot of iron and from the equation increase in the amount of Utazi leaf and Turmeric root component will lead to increase in the iron content of the tea blends.

Table 6 : Vitamin composition (mg/100g) of functional tea blends from Utazi leaf (*Gongronema latifolium)*, Nchanwu (*Ocimum gratissimum)* and Turmeric root (*Curcuma longa)*.

S/N	Sample Codes	Vitamin E Content	Vitamin C Content
1	AC	$0.58^{de} \pm 0.06$	$3.63^{a} \pm 0.35$
2	BC	$0.43^{bc} \pm 0.02$	$3.67^{a} \pm 0.57$
3	CD	$0.39^{bc} \pm 0.02$	$3.50^{a} \pm 0.61$
4	EA	$0.53^{d} \pm 0.02$	$3.57^{a} \pm 0.72$
5	EC	$0.37^{b} \pm 0.07$	$3.83^{a} \pm 0.84$
6	GB	$0.38^{bc} \pm 0.02$	$3.97^{a} \pm 0.61$
7	KF	$0.38^{b} \pm 0.04$	$4.10^{a} \pm 0.44$
8	HA	$0.31^{a} \pm 0.08$	$4.13^{a} \pm 0.38$
9	CF	$0.45^{c} \pm 0.03$	$3.90^{a} \pm 0.95$
10	DC	$0.52^{d} \pm 0.02$	$3.73^{a} \pm 0.47$
11	DA	$0.64^{e} \pm 0.03$	$3.63^{a} \pm 0.59$
12	JA	$0.37^{b} \pm 0.03$	$3.67^{a} \pm 0.64$
13	AB	$0.42^{bc} \pm 0.02$	$3.80^{a} \pm 0.72$
14	CA	$0.43^{b} \pm 0.02$	$3.67^{a} \pm 0.57$
15	GC	$0.45^{c} \pm 0.03$	$3.90^{a} \pm 0.95$
16	EF	$0.52^{d} \pm 0.02$	$3.73^{a} \pm 0.47$
17	LC (control)	$0.63^{e} \pm 0.03$	$3.03^{a} \pm 0.06$

Values are means of triplicate determinations ± Standard Deviation. Values in the same column bearing different superscript differ significantly ($P < 0.05$).

Vitamin E content of samples ranged from 0.31 to 0.64 mg/100g, with the lowest value recorded in sample HA (0.31 mg/100g) and the highest value recorded in sample DC (0.64 mg/100g). This result was in line with the findings of Enemor et al. [30] on utazi and recorded 0.54 mg/100g. Vitamins are organic substances which are essential for health, required in very small quantities and must be provided in diet because the body cannot synthesize them Takai, [32].

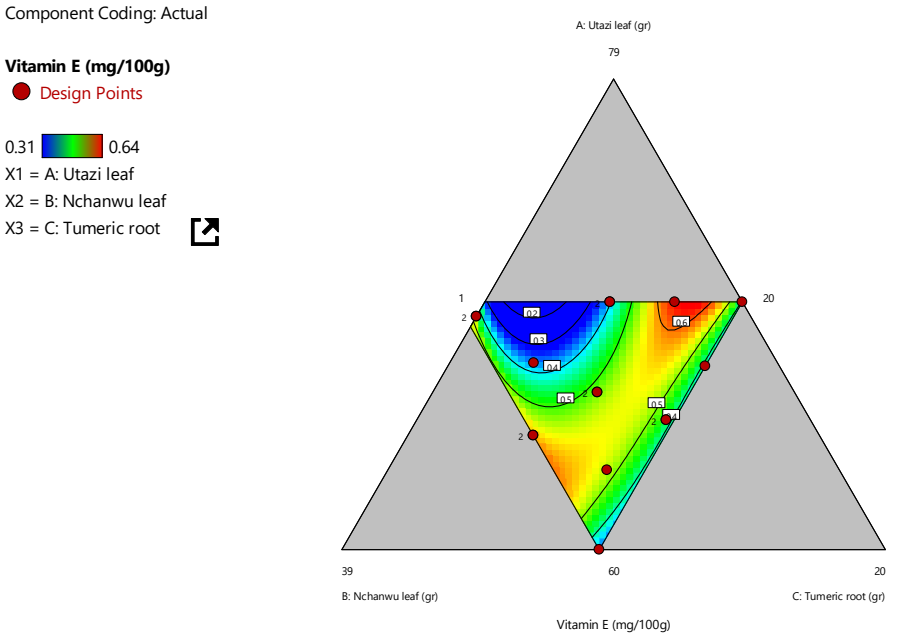

Figure 12**:** Contour plot of Vitamin E in the Functional tea blends

Vitamin E =A+B+C+AB+AC

Vitamin E = 0.33A+3.99B-11.03C-6.92AB+22.30AC…… Eq. 19.

Vitamin E content was modeled as shown in Eq. 19. Special Cubic model suggested by Mixture Design D-optimal ANOVA for vitamin E content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 12 shows the contour plot of vitamin E and from the equation increase in the amount of Turmeric root component will lead to decrease in the vitamin E content of the tea blends.

Vitamin C content ranged from 3.03 to 4.13 mg/100g, with sample LC having the lowest value (3.03 mg/100g) and sample HA having the highest value (4.13 mg/100g). However these finding was not consistent with work of Enemor et al. [30] on Utazi leave (15.84 mg/100g), this must be as a result of the processing steps in production. Vitamin C and E are very important antioxidants which protect the cell membranes from oxidative damage caused by free radical Guyton and Hall, [33]. Vitamin C has an antioxidant property and is therefore required for the maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine Button [34].

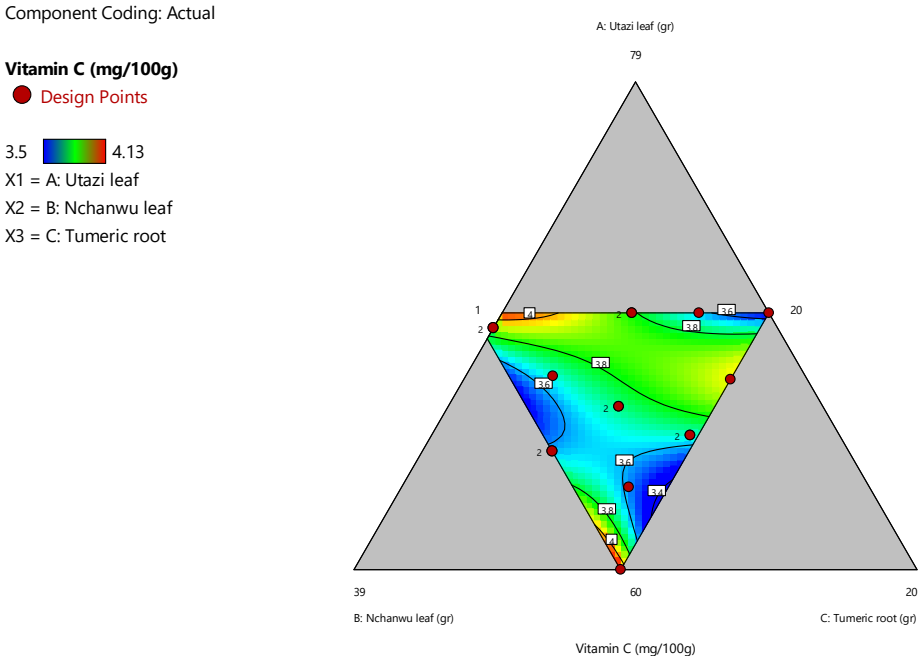

Figure 13**:** Contour plot of Vitamin C for the Functional tea blends.

VitaminC=A+B+C+AB+AC+BC+ABC+AB(A-B)+AC(A-C)+BC(B-C)

Vitamin C = -2.74A+8.17B-2.06C+4.85AB+24.43AC+3.06BC-26.02ABC+33.86AB(A-B)- 13.15AC(A-C)+3.12BC(B-C)…… Eq. 20

Vitamin C was modeled as shown in Eq. 20 Special Cubic model suggested by Mixture Design D-optimal ANOVA for vitamin C content. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 13 shows the contour plot of iron and from the equation increase in the amount of Nchanwu leaf component will lead to increase in the vitamin C content of the tea blends.

Table 7: DPPH scavenging activity (%) of functional tea from local spices mixture.

S/N	Sample Codes	DPPH Activities (%)
1	AC	$42.50^{b} \pm 3.54$
2	BC	$42.00^{b} \pm 1.41$
3	CD	$45.00^{b} \pm 2.83$
4	EA	$43.00^{b} \pm 1.41$
5	EC	$47.00^{b} \pm 2.83$
6	GB	$45.00^{b} \pm 4.24$
7	KF	$44.00^{b} \pm 1.41$
8	HA	$43.00^{b} \pm 4.24$
9	CF	$46.50^{b} \pm 0.71$
10	DC	$45.00^{b} \pm 0.00$
11	DA	$43.50^{b} \pm 0.71$
12	JA	$43.50^{b} \pm 0.71$
13	AB	$42.50^{b} \pm 3.54$
14	CA	$42.00^{b} \pm 1.41$
15	GC	$46.50^{b} \pm 0.71$
16	EF	$45.00^{b} \pm 0.00$
17	LC (control)	$36.50^{a} \pm 2.12$

Values are means of triplicate determinations ± Standard Deviation. Values in the same column bearing different superscript differ significantly ($P < 0.05$).

The DPPH scavenging effect ranged from 36.50 to 47.00%, with sample LC having the lowest value (36.50%) and sample EC having the highest value (47.00%). These were not consistent with the findings of Adekanle and Omozokpia [35] on Utazi leaf extract (17.94%). These entails that functional tea has high level of antioxidant when compared to the aforementioned work and the standard sample. Therefore the tea mixtures will reduce the effect of reactive oxygen species and prevent cancer, tumor, heart diseases, stroke, rheumatoid arthritis and diabetes Oloyede and Afolabi, [36]. Usually, higher total phenolic content leads to better DPPH radical scavenging activity (Ebrahimzadeh et al. [37].

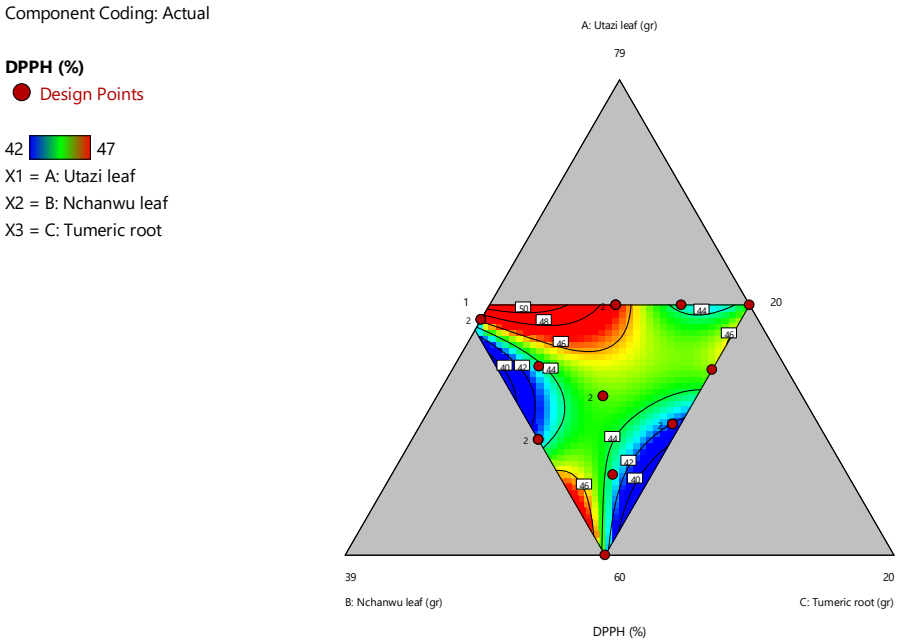

Figure 14**:** Contour plot of DPPH Activities of the Functional tea blends.

DPPH = A+B+C+AB+AC+BC+AB(A-B)+AC(A-C)+BC(B-C)

DPPH = 60.88A+14.10B-78.47C+38.81AB+222.61AC+281.18BC-385.80AB(A-B)+219.97AC(A-C)-414.86BC(B-C)…… Eq. 24.

DPPH activity was modeled as shown in Eq. 24. Special Cubic model suggested by Mixture Design D-optimal ANOVA for DPPH. The coefficient **A**, **B** and **C** represents Utazi leaf, Nchanwu leaf and Turmeric root respectively. Figure 14 shows the contour plot of DPPH and from the equation increase in the both Utazi and Nchanwu leaf component will bring about an increase in the DPPH activity in the tea blends.

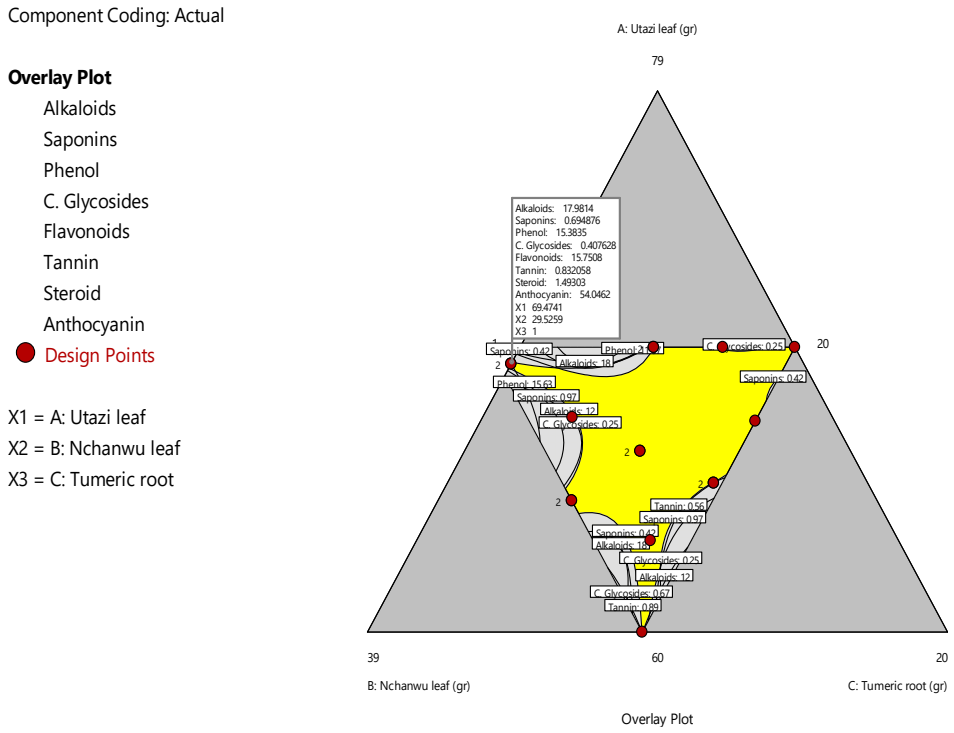

Figure 15**: Optimization for the Phytochemical composition of the Functional tea blend.**

Design expert response optimizer was used to carry out the optimization of the phytochemical composition. The responses were set as follows: Alkaloid (maximized), Saponin (in range), Phenol (maximized), Cardiac Glycoside (in range), Flavonoid (maximized), Tannin (in range), Steroid (in range), and Anthocyanin (maximized). The setup generated one solution for numerical optimization at maximum desirability of 0.97 for X_1 (69.47 g), X_2 (29.53 g) and X_3 (1 g) as Alkaloid = 17.98, Saponin = 0.69, Phenol = 15.38, C. Glycoside = 0.41, Flavonoid = 15.75, Tannin = 0.83, Steriod = 1.49 and Anthocyanin = 54.05.

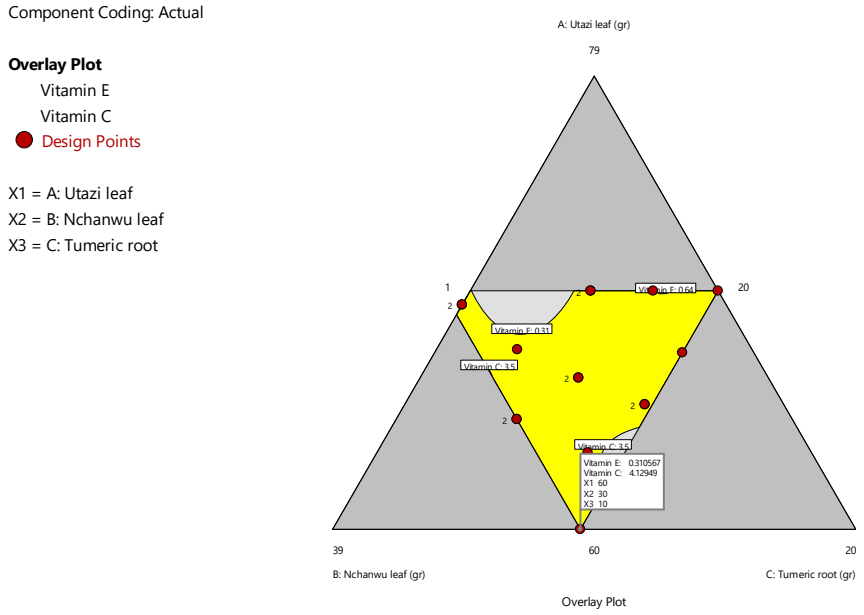

Figure 16**: Optimization for Vitamin content of the functional tea blends.**

Optimization for vitamin C and E were carried out using design expert response optimizer. The responses were set as follows: Vitamin E (in range), Vitamin C (maximized). The setup generated one solution for numerical optimization at maximum desirability of 1.0 for X_1 (60 g), X_2 (30 g), X_3 (10 g) as Vitamin E = 0.31 and Vitamin C = 4.13. This was result observed in sample HA.

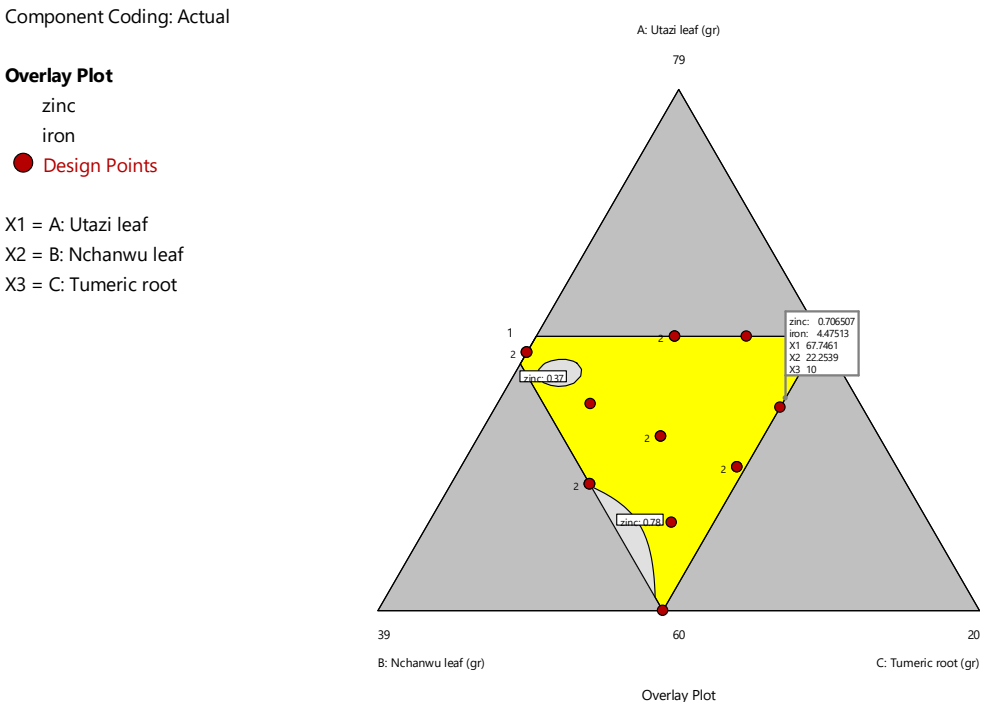

Figure17**: Optimization for the Mineral content of the Functional tea blends.**

To carry out the optimization for the mineral content, the design expert response optimizer was used. The responses were set as follows: Zinc (maximized), and Iron (maximized). The setup generated one solution for numerical optimization at maximum desirability of 0.81 for X_1 (67.75 g), X_2 (22.25 g) and X_3 (10 g) as Zinc = 0.71 and Iron = 4.48.

5.0 Conclusion

This study reveals that the mixture of Utazi leaf, Nchanwu leaf and Turmeric root, sourced locally produced a functional tea that is acceptable and nutritious, this implies that it can be taken as a meal, and it contains high level phytochemicals which assist in therapeutic functions, and it is also possess good antioxidant activity that scavenges free radicals. In addition, it contains minerals (zinc and iron) and vitamins (C and E) that ensure the maintenance of sound health. From this work the optimal blend of the raw material was obtained. The functional tea samples were good sources of antioxidant with scavenging activity of 47.00% in sample EC which had the composition (Utazi 70g, Nchawnu 24.62g, Turmeric 5.38g) and the phytochemical contents (alkaloids, phenol, anthocyanin, and flavonoid) make it suitable for management of ailments such as diabetes miletus type II and obesity.

5.1 Recommendation

From the findings the functional tea produced from Utazi leaf, Nchanwu leaf and Turmeric root is safe for consumption and is a natural source of antioxidant that is affordable and this can lead to product development and creation of employment and the synergy produced from the blends possess medicinal and therapeutic properties. From the optimization phytochemical content the optimal blend for the tea is Utazi leaves (69.47 g), Nchanwu leaves (29.53 g) and Tumeric root (1 g), this was similar with the proportion of sample GC having Utazi leaves (69.41 g), Nchanwu leaves (29.59 g) and Tumeric root (1 g). The processing steps which include oxidation for 24 hr at 28-30^{o}C and oven drying at 60^{o}C for 30 min assisted in reducing the anti-nutrient in the raw materials.

Disclaimer

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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