Phytochemical, Antioxidant Properties and Volatile Compounds of Tisanes Prepared From Aidan (Tetrapleura tetraptera) Fruit and Uziza (Piper guineense) Seeds

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ABSTRACT

Background: *Some local herbs are still underexploited especially in the form of tisanes.*

Objective: The present study aims to assess the potentials of Aidan (Tetrapleura tetraptera) fruit and Uziza (Piper guineense) seeds in the production of tisanes and evaluate the phytochemical, antioxidant and volatile compounds of the tisanes.

Methods: *Uziza seeds and Aidan fruits were sorted, washed, dried and milled before packaging in tea bags (50 g per tea bag) while a commercial lemon grass and ginger tisane was used as control. The tisane infusions were evaluated for their physicochemical, phytochemical, antioxidant, sensory properties and volatile compounds using standard methods.*

Results: *The pH of the tisane extracts were slightly acidic (5.30 to 6.54), the saponin contents ranged from 0.05-0.14%, tannin from 22.91-24.33%, phenol from 1.75-2.92% and alkaloid from 3.15-5.39%. The ferric reducing antioxidant power (FRAP) of uziza tisane (73.72mg/100g) was significantly higher than that of aidan fruit (49.29mg/100g) and the control (57.26mg/100g). Forty eight volatile compounds including hydrocarbons (mainly terpenes), aldehydes and alcohols were identified in the tisane samples amongst which were piperine identified in uziza (0.66%) and Apiol in both uziza and aidan fruit (5.43%). Uziza tisane was the least preferred in terms of the sensory parameters while the control was the most preferred.*

Conclusion: *The study showed that uziza and aidan fruit can be used in the production of tisanes with health promoting potentials.*

Keywords: Tisane, Aidan fruit, *Uziza* seeds, antioxidant properties, volatile compounds.

INTRODUCTION

The World Health Organization capitalized on the use of traditional medicines including herbal medicines in its 2014– 2023 strategy, of keeping populations healthy by providing access to effective and affordable alternatives to medicine, and providing healthcare choices coherent with people's cultural practices [1]. In recent times, there is renewed interest in tea resulting from growing consumer awareness of the health benefits derived from tea consumption [2]. Herbal tea or tisane refers to a beverage not made from *Camellia sinesis* infusions; but made from the infusion in hot water of flowers, leaves, seeds or roots of herbs, spices or any other plant

material generally known to possess medicinal values. Tisanes are popular because of their fragrance, antioxidant properties and therapeutic applications. Most herbal teas may consist of one main herbal ingredient or a blend of herbal ingredients, intended to bring about a specific purpose such as relaxation, rejuvenation and relief from a specific condition amongst other things [3]. Therefore, it is of great importance to explore the potential of indigenous plants, which have not been properly researched such as Aidan fruit (*T. tetraptera*), and *Uziza* seeds (*P. guneense*) in the development of herbal tea. Aidan fruit (*T. tetraptera*) is a species of covering plant in the pea family native of western Africa [4]. The Aidan fruit tree has many uses; its sweet fragrance is highly valued. The fruit is used to spice dishes and its bark is used for medicinal purposes. The distinct fragrance of the fruit is attributed to its essential oil content [5]. It is used extensively in soup for nursing mothers to prevent post-partum contractions and intestinal disorders especially stomach ulceration [6][7][8]. The fruit has wide application in Nigerian folk medicine in the management of some ailments including diabetes mellitus, arthritis, hypertension, epilepsy, asthma etc. [9][5].

Piper guneense (*Uziza*) can be used as a local spice in Nigerian dishes to give a hot, slightly bitter and pungent aroma to the food. It has nutritional, culinary, medicinal and insecticidal value. It is said to be therapeutically useful in the management of convulsion, leprosy, stomach ache, inflammation and/or rheumatoid pains, cough and loss of appetite [10]. *Piper guineense* is added to food meant for nursing mothers as a medicinal spice and among postpartum women as it is claimed that it assists in the contraction of the uterus [11].

Indigenous herbs are in general heavily under-exploited despite their huge health potentials [12]. Therefore, there is a great need to explore the potential of these indigenous plants in the development of tisanes, which are easy to consume so that the beneficial effects can be harnessed and the cost of importing tisanes from other countries would be reduced. The objective of this study was to produce and evaluate herbal tea from Aidan fruit (*T. tetraptera*) and *Uziza* seeds (*P. guineense*). The findings of this research work will benefit nursing mothers and tea-producing industries in diversifying their products.

2.0 MATERIALS AND METHODS

2.1 MATERIALS

Freshly dried Aidan fruit and *Uziza* seeds were purchased from Ndoro market, Ikwuano L.G.A of Abia state, Nigeria.

2.2 PREPARATION OF TISANES

Uziza seeds and Aidan fruits were sorted and washed carefully with clean water and dried at 60^{0}C in an oven for 12 hours after which the samples were milled with a hammer mill and packaged (50 g per tea bag) differently in tea bags as described by [13]. A commercial lemon and ginger tisane was used as control. Each tea bag was infused with 100mL of hot water (80°C) and steeped for 10 mins. The extracts were stored at 4°C until required for analysis. Analyses of the tea extracts were done in triplicate.

2.3 PHYSICO-CHEMICAL ANALYSIS

2.3.1 pH Determination

The pH of the tea extracts were determined following the method described by [14]. The pH reading was done by direct measurement using an electronic digital pH meter (model Hi-2210). The pH meter was standardized with a buffer solution before the readings were taken. The electrodes were dipped into the tea and the readings were allowed to stabilize and then recorded.

2.3.2 Determination of Total Titratable Acidity (TTA)

The AOAC official method [14] was used to determine the titratable acidity.

Ten (10) milliliters of the tea extract was titrated with 0.1 NaOH using phenolphthalein solution as indicator. Titratable acidity was calculated with the following formula.

$$\text{TTA (\%)} = \frac{\text{V} \times \text{N}}{\text{volume of sample}}$$

Where V = titre value obtained

N = normality of the titrant

2.3.3 Determination of Moisture Content

The moisture content of the tisane samples was determined according to the standard method of [14]. The petri dishes were washed thoroughly and afterwards dried in the oven at 100^{0}C for 1hour. The weight (W1) was taken when cooled, 10grams of the sample was weighed into the petri-dish and the total weight (W2) was taken before and during drying at 100^{0}C until a constant

weight (W3) was obtained.

$$\% \text{ moisture content} = \frac{W2-W3}{W2-W1}$$

Where W1 = Initial weight of empty dish
W2 = weight of dish + weight of sample before drying
W3 = weight of dish + weight of sample after drying

2.4 PHYTOCHEMICAL ANALYSIS

2.4.1 Determination of Tannin Content

The method used to determine the tannin component was the Folin-Denis spectrophotometric method described by [15] and [16]. Exactly 1gram of each sample was weighed and dispersed in 10millilitre distilled water and agitated. This was left to stand for 30 minutes at room temperature, being shaken every 5 minutes after which it was centrifuged and 2.5millilitre of the supernatant (extract) was dispersed into a 50millilitre volumetric flask. Similarly, 2.5millilitre of standard tannic acid solution was dispersed into a separate 50millilitre flask. A 1.0millilitre Folin-Denis reagent was measured into each flask, followed by 2.5millilitre of Na_2CO_3 solution. The mixture was diluted to mark in the flask (50millilitre), and incubated for 90 mins at room temperature. The absorbance was measured at 250nm in a UV/Visible spectrophotometer (model V1700, product ID; 201507005 made in UK). Readings were taken with the reagent blank at zero.
The tannin content is given as follows;
%Tannin=An/As x C x 100/W x Vf x Va
Where;
An=absorbance of 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.

2.4.2 Determination of Total Saponin Content

The double solvent extraction gravimetric method was used [17]. Exactly 5g of the ground sample was mixed with 50ml of 20% aqueous ethanol solution and incubated for 12h at temperature of 55°C with constant agitation after which it was filtered through whatman No. 42 filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 mins and the extracts were weighed together and reduced to 40mls by evaporation, transferred to a separating funnel where 40ml of ether was added to it and mixed. They were partitioned; the aqueous layer was reserved while the other layer was discarded. The aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 by drop wise addition of NaOH solution. The saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of butanol. The precipitate was washed with 5% NaCl solution and evaporated to dryness in a previously weighed evaporation dish. The saponin was dried in the oven at 60°C, cooled in
a desiccator and re-weighed.

$$\% \text{ saponin} = \frac{W2-W1}{W}$$

W = weight of sample used
W1 = weight of empty evaporation dish
W2 = weight of dish

2.4.3 Determination of Total Flavonoid Content

The method used to determine the total flavonoid content was that described by [15]. The total flavonoid content was determined colorimetrically using aluminum chloride ($AlCl_3 6H_2O$) solution and quercetin was used as a reference to produce a standard curve. Exactly 1millilitre of the tisane sample extract was placed in a 10millilitre volumetric flask containing 5millilitre of distilled water and 0.3millilitre of 5% sodium nitrite was added and mixed. After 5mins, 0.3millilitre of 10% aluminum chloride solution ($AlCl_3 6H_2O$) was added and the mixture was allowed to stand for another 6minutes after which 2millilitres of 1M sodium hydroxide was added and properly mixed. Absorbance of the reaction mixture was read at 510 nm after 30 minutes with a spectrophotometer (model V1700, product ID; 201507005 made in UK).

2.4.4 Determination of the Total Phenolic Content (TPC)

The total phenolic content of the samples was determined using Folin-Ciocalteau colorimetric method as described by [15]. An aliquot of 0.3ml of the sample extract was mixed with 2.25millilitre of Folin-Ciocalteu phenol reagent. After 5 mins, 6% sodium carbonate (2.25mL) was added and the mixture was allowed to stand at room temperature for 90 mins. The absorbance of the mixture was measured at 725 nanometer. Standard calibration curve for garlic acid in the

range of 0-200 lg/ml is prepared in the same manner and results are expressed as gallic acid equivalent (GAC) per gram of extract.
TPC=C*V/W
C= concentration of gallic acid calculated from calibration curve in mg/ml
V=volume of extract in ml
W=weight of plant ethanoic extract in gram.
The total phenolic content of the extracts are expressed as mg of gallic acid equivalents, (mg GAE/ml).

2.4.5 Determination of Alkaloid Content

The method that was used to determine the alkaloid content of the samples was the gravimetric method of [17]. About 10 gram of the tisane samples were weighed out and dispersed into 50millilitres of 10% acetic acid solution ethanol. The mixture was stirred well and allowed to stand for 4 hours before it was filtered. The filtrate was evaporated to one quarter of its original volume. Drop wise concentration of NH_4OH was added to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper and washed with 1% NH_4OH solution. The precipitate was dried in the oven at 60°C for 30 mins and reweighed. By weight difference, the weight of the alkaloid is determined and expressed as a percentage of the sample weight analyzed. Given by the formula:

$$\%\text{Alkaloid} = \frac{W2-W1 \times 100}{W}$$

Where:
W = weight of sample
W1 = weight of empty filter paper
W2 = weight of paper plus precipitate.

2.5 ANTIOXIDANT ASSAY

2.5.1 Determination of Terpenoid Content

The method used for the terpenoid content determination was that described by [15].
The gravimetric method was used in determination of the steroid content, where 0.5 grams of the samples were weighed inside a beaker and 10millilitre of ethanol was added, then the mixture was thoroughly mixed and left to stay overnight after which it was filtered using a filter paper. The filtrate was poured into a separating funnel and extracted with petroleum spirit. The ethanolic extract obtained was transferred into pre-weighed glass dishes and dried at 80°C for complete evaporation. Afterwards, the dishes were weighed.

$$\%\ \text{Terpenoid} = \frac{W2-W1 \times 100}{W0}$$

Where:
W_1 = weight of dish + residue after drying
W_2 = weight of dish + residue before drying

2.5.2 Determination of Steroid Content

The method used for the steroid content determination was that described by [15].
The spectrophotometric method was used in determination of the steroid content. Exactly 1gram of the sample was macerated with 20millilitres of ethanol. The solution was filtered using a filter paper, where 2millilitres of the filtrate was pipetted out and 2millilitres of cholesterol colour reagent was added. The solution was left to stand for 30minutes after which the absorbance of the solution was taken at 550nanometer.

$$\text{Concentration} = \frac{Absorbance\ of\ sample \times DF}{Gradient\ factor}$$

2.5.3 Ferric Reducing Antioxidant Power (FRAP) ASSAY

The method used for the determination of FRAP was that described by [15]. Fresh FRAP (Ferric reducing antioxidant power) reagent was prepared by mixing 300mM acetate buffer (100mL), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution (10mL) and 20 mM $FeCl_3.6H_2O$ (10mL) solution. It was kept warm at 37°C until ready for use. The 300 mM acetate pH 3.6 was prepared by dissolving 3.1grams sodium acetate trihydrate in 500millilitres distilled water, then 16millilitres acetic acid was added and made up to mark of 1 liter with distilled water and checked for its pH. The 10mM TPTZ solution was prepared in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ was prepared in distilled water. The calibration water was prepared in the range of 0-250 lg/mL using Trolox as the standard. The standard solution (150 $_{u}$L) and sample extract (150 $_{u}$L) were allowed to react with FRAP solution (2850 $_{u}$L) in different test tubes for 30 minutes in the dark. The coloured solution (ferrous tripyridyltriazine complex) of standard and sample at 593nm was read.
% scavenging activity= (Ca-Sa/Ca) x 100
Ca (absorbance of control), Sa (absorbance of sample)

The concentration of FRAP content in the extract was reported as mg Trolox equivalent (TE)/g extract. Calibration curve was obtained by plotting % inhibition against trolox standard concentration.

2.6. GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS)

The method of [18] was used in the identification of volatile compounds present in Aidan fruits and *Uziza* seed tea extracts. Compounds present in the extract were identified by GC-MS analysis (GC-MS-QP2010 PLUS Shimadzu, Japan) on Elite 5Ms column 20m long and 0.18μ internal diameter. The temperature was programmed from 200°C to 300°C at a rate of 4°C. The carrier gas was Helium with a constant flow at 1ml/min. Mass method used was Electron ionization voltage of (EI+) 70eV for m/z value 50 to 300 with a scan time of 0.3sec and the interscan delay of 0.1sec. The operations were:
Oven Temperature - 80°C
Injection Temperature- 250°C
Injection Mode- Split
Injection Port Dwell Time- 0.3sec.
Pressure- 108.0kPa
Linear Velocity- 46.3cm/sec.

2.7 SENSORY EVALUATION

A teabag containing 50g each of the tisane samples was infused in 100ml of hot (80°C) water. Sensory evaluation of the coded tisane infusions was carried out according to the method described by [19]. Untrained panelists composed of twenty (20) staff and students of Michael Okpara University of Agriculture, Umudike, Nigeria were asked to score the appearance, taste, mouth-feel, after-taste, aroma and general acceptability of the tisane infusions using the nine (9) point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely).

2.8 STATISTICAL ANALYSIS

All analyses were conducted in triplicates and data obtained were subjected to analysis of variance (ANOVA) using SPSS version 16. Differences among the means of the investigated parameters were separated using Duncan's new multiple range test at 5% probability.

3.0 RESULTS

3.1 Physicochemical properties of tisanes from aidan fruit and *uziza* seeds

The physicochemical properties of tisane produced from Aidan fruits (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*) are presented in Table 1. The pH of the samples ranged between 5.30 (Control) to 6.54 (*Uziza* seed tisane) which was within weakly acidic range. The TTA of the tisane samples followed a different trend from pH as tisanes produced from Aidan fruits had higher TTA values (0.014 %) relative to those produced from *uziza* seeds (0.013 %). It was also observed that the control had higher TTA value (0.016 %) than the tisanes from Aidan fruit and *uziza* seeds. The total solid content of the tisane samples ranged from 0.80 (control) to 3.70% (aidan fruit tisane). Aidan fruit tisane had the highest total solid content (3.70%) followed by that from *uziza* seeds (1.70%).

Table 1: Physicochemical properties of tisane produced from Aidan fruit (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*).

Sample	Moisture (%)	pH	TTA (%)	TS (%)
100 % Aidan fruit	$96.30^{c}\pm0.00$	$5.45^{b}\pm0.00$	$0.014^{b}\pm0.001$	$3.70^{a}\pm0.00$
100 % *Uziza* seeds	$98.30^{b}\pm0.00$	$6.54^{a}\pm0.00$	$0.013^{b}\pm0.000$	$1.70^{b}\pm0.00$
ginger and lemon tea (control)	$99.20^{a}\pm0.14$	$5.30^{c}\pm0.04$	$0.016^{a}\pm0.000$	$0.80^{c}\pm0.14$

Values are means ± standard deviations of duplicate determinations. Two means along the same column with different superscripts are significantly different ($p<0.05$).

Table 2: Phytochemical concentration of tisane produced from Aidan fruits (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*)

Phytochemicals	Tisane from Uziza seeds	Tisane from Aidan fruit	Control
Saponin (%)	$0.05^{b}\pm0.01$	$0.12^{a}\pm0.00$	$0.14^{a}\pm0.01$
Tannin (%)	$22.91^{a}\pm0.95$	$23.38^{a}\pm0.00$	$24.33^{a}\pm0.41$
Flavonoid (%)	$115.35^{a}\pm0.50$	$103.22^{b}\pm0.50$	$92.86^{c}\pm0.00$
Phenol (mgGAE/ml)	$1.75^{c}\pm0.00$	$2.38^{b}\pm0.00$	$2.92^{a}\pm0.01$
Alkaloid (%)	$3.15^{c}\pm0.08$	$3.99^{b}\pm0.17$	$5.39^{a}\pm0.20$

Values are means ± standard deviations of triplicate determinations. Means across the same row with different superscripts are significantly different ($p<0.05$).

Table 3: Anti-oxidant assay of tisane produced from Aidan fruits (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*)

Sample	FRAP (mg/100g)	Steroid (mg/100g)	Terpenoid (mg/100g)
Tisane from uziza seeds	$73.72^{a}\pm0.10$	$1.78^{a}\pm0.02$	$1.30^{a}\pm0.02$
Tisane from Aidan fruit	$49.29^{c}\pm0.14$	$1.30^{c}\pm0.02$	$0.05^{c}\pm0.00$
Control	$57.26^{b}\pm0.03$	$1.47^{b}\pm0.01$	$0.25^{b}\pm0.01$

Values are means ± standard deviations of triplicate determinations. Means along the same column with different superscripts are significantly different ($p<0.05$).
Key: FRAP = Ferric Reducing Antioxidant Power.

3.2 Phytochemical content of tisane produced from *Aidan* fruits and *Uziza* seeds

The phytochemical content of the tisanes are shown in Table 2. Aidan fruit tisane had higher saponin content (0.12 %) and is not significantly ($p>0.05$) different from the control (0.14 %), while *uziza* seed tisane had the lowest saponin content (0.05 %). The tannin concentration of *uziza* tisane (22.91 %) and Aidan fruit tisane (23.38 %) was not significantly different from that of the control (24.23 %).

The result showed that *uziza* tisane had the highest flavonoid content (115.35 %) followed by Aidan fruit tisane (103.22 %) while the control had the least flavonoid content (92.86 %). The total phenol content of *uziza* and Aidan fruit tisane was 1.75 and 2.38 % respectively and differed significantly. The alkaloid content of *uziza* tisane (3.15 %) and Aidan fruit tisane (3.99 %) were significantly ($p<0.05$) lower than that of the control (5.39 %).

3.3 Antioxidant assay of tisane produced from Aidan fruits and *Uziza* seeds

The anti-oxidant assay of tisane produced from aidan fruits (*Tetrapleura teraptera*) and *uziza* seeds (*Piper guineense*) is presented in Table 3. The FRAP value of *Uziza* tisane (73.72 mg/100g) was significantly ($p<0.05$) higher compared with that of the control (57.26 mg/100g) while FRAP value of Aidan fruit tisane (49.29 mg/100g) was significantly ($p<0.05$) lower than that of the control. The steroid content of *uziza* tisane was 1.78 mg/100g which was significantly ($p<0.05$) higher than that of aidan fruit tisane (1.30 mg/100g) and the control (1.47 mg/100g). The concentration of terpenoid was observed to be significantly ($p<0.05$) highest in *Uziza* tisane (1.30 mg/100g) followed by the control (0.25 mg/100g) while aidan fruit tisane recorded the least concentration (0.05 mg/100g).

Table 4: Concentrations of volatile compounds in tisane produced from *Uziza* seeds, Aidan Fruits and Ginger and Lemon Tea.

S/N	Retention Time (minutes)	Compounds	Formula	Peak Area (%) *Uziza* seeds tisane	Aidan fruit tisane	Ginger and lemon tea
		Hydrocarbons				
1	4.062	Decane	$C_{10}H_{22}$	-	1.87	-
2	10.072	Tridecane	$C_{13}H_{28}$	-	2.23	-
3	11.390	Copaene	$C_{15}H_{24}$	3.74	5.48	
4	11.551	1-Tetradecene	$C_{14}H_{28}$	-	7.13	1.45
5	11.576	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	$C_{15}H_{24}$	3.27		
6	11.668	Tetradecane	$C_{14}H_{30}$	-	1.64	-
7	12.075	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene-	$C_{15}H_{24}$	-	17.66	-
8	12.078	Caryophyllene	$C_{15}H_{24}$	13.63	-	-
9	12.168	γ-Elemene	$C_{15}H_{24}$	2.88	3.49	
10	12.432	cis-β-Farnesene	$C_{15}H_{24}$	-	3.86	1.50
11	12.434	(E)-β-Farnesene	$C_{15}H_{24}$	3.78	-	-
12	12.484	α-Cubebene	$C_{15}H_{24}$	1.58	1.76	-
13	12.590	Humulene	$C_{15}H_{24}$	2.19	3.03	
14	12.816	α-Guaiene	$C_{15}H_{24}$	2.00	2.74	-
15	12.863	Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	-	-	7.27
16	12.938	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	-	7.54	-
17	12.940	Germacene D	$C_{15}H_{24}$	7.89	-	-
18	13.052	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-	$C_{15}H_{24}$	-	-	2.78
19	13.057	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,1α,4aβ,8aα)-	$C_{15}H_{24}$	-	4.38	-
20	13.148	Alloaromadendrene	$C_{15}H_{24}$	1.82	2.78	-
21	13.236	β-Bisabolene	$C_{15}H_{24}$	6.61	6.80	3.95
22	13.455	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-	$C_{15}H_{24}$	1.81	2.04	3.86
23	13.989	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	$C_{15}H_{24}$	0.74	-	-
24	14.273	Cetene	$C_{16}H_{32}$	-	2.49	0.78
25	15.174	Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl-	$C_{15}H_{24}$	5.93	5.43	9 2.26
26	15.573	(E)-β-Farnesene	$C_{15}H_{24}$	3.78	-	2.20
27	27.370	Squalene	$C_{30}H_{50}$			12.28
28	27.976	Nonacosane	$C_{29}H_{60}$	-	2.02	2.18
29	28.186	Piperine	$C_{17}H_{19}NO_3$	0.66	-	-
		Aldehyde				
30	8.312	Decanal	$C_{10}H_{20}O$	-	-	3.26
		Alcohol				
31	29.866	Vitamin E	$C_{29}H_{50}O_2$	-	-	2.12
32	14.639	Apiol	$C_{12}H_{14}O_4$	5.93	5.43	
33	14.193	(2E,4S,7E)-4-isopropyl-1,7-dimethylcyclodeca-2,7-dienol	$C_{15}H_{26}O$	0.90	-	
		Others				
34	13.391	1,3-Benzodioxole,4-methoxy-6-(2-propenyl)-	$C_{11}H_{12}O_3$	2.48	2.46	-
35	13.677	Benzene,1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	1.80	1.38	-
36	13.793	Cyclohexanemethanol,4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-	$C_{15}H_{26}O$	0.58		-
37	13.889	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl	$C_{15}H_{26}O$	0.71	-	
38	14.912	2-Butanone,4-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{14}O_3$	0.90	-	12.30
39	22.239	2 -Propanone,1-(4-hydroxy-3-methoxyphenyl)-	$C_{10}H_{12}O_3$	-	-	1.73
40	22.987	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	$C_{10}H_{16}O$	-	-	13.18
41	24.094	3,4-Methylenedioxyphenyl acetone	$C_{10}H_{10}O$	0.87	-	-
42	24.949	2-Butanone,4-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{14}O_3$	-	-	2.89
43	25.493	Isoxaben	$C_{18}H_{24}N_2O_4$	0.91	-	-
44	25.839	1,3-Benzodioxole,5-propyl-	$C_{10}H_{12}O_2$	1.71	-	-
45	26.065	Adamantane,1-chloro-	$C_{10}H_{15}Cl$	9.86	-	-
46	27.353	Isoxaben	$C_{18}H_{24}N_2O_4$	18.93	11.76	
47	26.673	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	$C_{10}H_{16}O$	-	-	3.32
48	17.369	Caffeine	$C_8H_{10}N_4O_2$	-	-	18.66

Table 5: Sensory scores of tisane produced from blends of Aidan fruits (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*)

Sample	Taste	Mouth-feel	Appearance	After-taste	Aroma	General acceptability
Control	$6.8^{a}\pm1.03$	$7.1^{a}\pm1.10$	$7.7^{a}\pm1.05$	$6.9^{a}\pm1.19$	$6.7^{a}\pm0.67$	$7.2^{a}\pm0.78$
Aidan fruit tisane	$6.3^{ab}\pm1.15$	$7.0^{a}\pm1.56$	$7.6^{a}\pm0.96$	$6.5^{a}\pm1.64$	$6.2^{ab}\pm1.81$	$6.6^{ab}\pm1.77$
Uziza tisane	$5.0^{b}\pm1.05$	$5.5^{b}\pm0.84$	$5.8^{b}\pm0.78$	$5.4^{a}\pm2.01$	$5.3^{b}\pm1.41$	$6.2^{b}\pm0.91$

Values are means ± standard deviations of duplicate determinations. Two means along the same column with different superscripts are significantly different ($p<0.05$).

4.0 DISCUSSION

The pH of the tisane samples is similar to the range (5.35 to 5.81) reported for tisane from *Chromolaena odorota* by [20]. The lower pH of the tisane produced from Aidan fruits (5.45) and the control (5.30) relative to that produced from *uziza* seed suggests better storage stability of bioactive compounds in the tisanes [21][22]. The TTA values of the tisane samples were quite lower than the range (0.092 to 0.174 %) reported for TTA of ready-to-drink flavoured-coloured commercial teas by [23]. The more acidic a drink, the less susceptible it is to bacterial action [24]. According to [25], total titratable acidity has an impact on the flavour of food. The total solid content of the tisane infusions is inversely related to its moisture content and is equivalent to extractable components of the tisane.

The saponin concentrations were quite lower than 2.5-3.50% reported for fresh *Moringa oleifera* tea [26]. Saponins have been reported to have anti-carcinogenic properties, immune modulation and cholesterol lowering activities [27]. The tannin content of the tisane samples studied was higher than 0.76-1.32 mg/100 mL reported for tisanes from *Chromolaena odorota* by [20]. Tisanes with high tannin content are usually astringent and could be used for the treatment of different ailments [28]. The flavonoid content of the tisanes was quite higher than the range (5 to 15 %) reported for different brands of tea by [29]. Flavonoids are strong anti-oxidants with antimicrobial and anti-inflammatory properties [30][26]. This implies that *uziza* and aidan fruit tisanes are likely to possess anti-oxidant and anti-inflammatory properties as well as anti-carcinogenic and anti-mutagenic activities. Phenolic compounds refer to a large group of secondary metabolites in natural products involved in an extensive range of bioactivities ameliorating risks of oxidative stress-related diseases [31][32]. Some phenolic compounds have several health benefits, like cardiovascular protection and anticancer effects, which are associated with their strong antioxidant activities [33][34]. Therefore, consumption of the tisanes in this study would have some health promoting effects on the body. Phenols are also the main source of bitterness and astringency of tea infusions [35]. The alkaloid content of the tisane samples were higher than the values reported for black tisane (0.92 mg/100ml) and green tisane (0.61 mg/100ml) by [20]. Alkaloids have been reported to have a powerful effect on animal physiology and play some metabolic roles and control development in living systems [36][37][38].

Ferric Reducing Antioxidant Power (FRAP) assay measures the reducing ability of antioxidants against the oxidative effect of reactive oxygen species [20]. *Uziza* tisane could compete favourably with the control in terms of the ability to scavenge free radicals. The high content of steroids and terpenoids in *uziza* shows that they could have antioxidant, anticonvulsant, antiulcer, anti-inflammatory, antiseptic, antitumor, antiviral, analgesic, antihypertensive, antibacterial, and therapeutic anti-diabetic properties [39][40].

Volatile compounds are largely responsible for the flavor and aroma of tisanes. Similar findings of terpene dominance in teas have been reported by [41]. According to [42], terpenes are a major chemical group and are important contributors to

aroma of tea. A similar observation of alpha-copaene and beta-elemene in fruit (berries) of *P. guineense* as observed in the tisanes studied was made by [43]. Apiol found in tisanes from *uziza* seeds and aidan fruit has been reported to increase the tone and strength of miometral contraction, reduce the tone of vessels and cause necrosis of placental tissue leading to abortion [44]. This justifies why these tisanes should be used for postnatal contractions and never at the prenatal stage.

The tisane from *uziza* was the least preferred in terms of the sensory properties. This could be due to the pungency of the *uziza* tisane which is not accepted by all. However, generally, all the tisanes were acceptable by the panelists since none had a sensory score below 5 which would have been in the dislike region of the 9-point hedonic scale.

5.0 CONCLUSION

The findings of this study revealed that tisane with acceptable quality can be produced from Aidan fruit (*Tetrapleura teraptera*) and *Uziza* seeds (*Piper guineense*) with antioxidant and health promoting potentials. The *uziza* and *aidan* fruit tisanes are convenient beverages recommended for use at the post-natal stage and never at the prenatal stage to encourage contraction of the uterine walls.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests.

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