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Phytochemical, Nutritional and Anti-Nutritional Analyses of Ruzu Herbal Bitters

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Abstract:
Background: *The phytochemical, nutritional and antinutritional analyses of Ruzu herbal bitters (RHB) were investigated. RHB is a polyherbal mixture with therapeutic effects comprising of three plants - Curculigopilosa root, Citrullus colocynthis bark and Uvariachamae stem.*
Materials and methods: *Standard methods were used for the analyses. The methods of Trease and Evans (1989) and Harborne (1973) were used for qualitative phytochemical analysis; AOAC (2000) for proximate composition, amongst others.*
Results: *The phytochemical analysis of RHB showed that the product is rich in saponins (0.77 mg/ml), alkaloids (0.76 mg/ml), flavonoids (0.73 mM), and cardiac glycosides (0.32 mM), while phenols (0.09 mg/ml), steroids (0.09 mg/ml) and tannins (0.01 mg/ml) are least amongst the phytochemicals analyzed. Proximate analysis showed that the product has high ash content (57.2 %) and reasonable amounts of moisture (14.16 %), carbohydrate (12.35 %), fibre (11.13 %) and protein (4.87 %) with low percentage composition of fat (0.60 %). The vitamins content, in a decreasing order of abundance, includes vitamins K, D, C, A, B3, B2, E and B1. The mineral content includes Mg, K, Fe, Na, Cr, Cd, Mn and Pb. RHB contains 18 standard amino acids, with asparagine and glutamine missing which could be lost during acid hydrolysis. The antinutritional analysis showed percentage composition of saponins (76.73), cardiac glycosides (36.13), phytates (1.33), oxalates (1.15), alkaloids (0.93), cyanogenic glycosides (0.80) and tannins (0.01).*
Conclusion: *The presence of these secondary metabolites in RHB is an indication that it has medicinal effects as some of them serve as antioxidants and antibiotics, amongst others. The nutrients and antinutrients present in the product will respectively add to the bulk of nutrients in the body and mediate in nutrients availability.*
Key words: *Ruzu herbal bitters (RHB),Curculigopilosa root, Citrullus colocynthis bark, Uvariachama, Proximate analysis, Antinutritional analysis.*

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I. Introduction

Plants are amongst the nature's gift to humanity. Their importance ranges from food to medicine. Hence, apart from serving as the primary producers in the food chain, plants are the primary source of therapeutic agents. This is as a result of the secondary metabolites also known as phytochemicals they contain. In high concentrations at times, some phytochemicals act as anti-nutrients, affecting the absorption or and, of course, the utilization of some nutrients.

Phytochemicals are plant-derived chemicals which are important to human health and help to ameliorate diseases. The term is generally used to refer to those chemicals that may have biological significance, like antioxidants, antimicrobial and antiviral activities, but are not established as essential nutrients which when in excess could be detrimental. It is pertinent to state that in as much as they help to strengthen the body defense mechanism serving mostly as antioxidants, moderate consumption is highly recommended so that they will not serve as anti-nutrient to the body (Ifemeje*et al.,* 2014). Phytochemicals have the potential to affect diseases such as cancer, stroke and metabolic syndrome and those caused by microorganisms (Saidulu*et al.,* 2014). Phytochemicals such as vitamins, terpenoids, phenolic acids, lignins, stibenes, tannin, flavonoids, quinones, coumarins. alkaloids, amines and other metabolites are rich in antioxidant activity (Cai *et al.,* 2003). It has been shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antituumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Sala *et al.,* 2002). In terms of nutritional values, different parts of many plants serve as good sources of nutrients such as carbohydrates, proteins, fats minerals and vitamins; thereby adding to the 'pool' of nutrients and enhancing their therapeutic efficacy.

It has become a common traditional practice in some cases, that a combination of plants or their extracts is used in the treatment of certain ailments with the believe by the herbalists that the individual plants

contain different therapeutic agents in which when combined together will give a better therapeutic efficacy for a particular disease or multiple diseases than that of a single plant. Therefore, most herbal preparations work in synergy, hence the emergence of polyherbal mixtures.

Ruzu herbal bitters (RHB) is a polyherbal mixture, produced by Ruzu Natural Health Product and Services, Nigeria, with a NAFDAC Registration Number: A7-1102L. The polyherbal mixture is made up of three different plants: 20 % *Uvariachamae*(bush banana), 40 % *Citrullus colocynthis*(bitter apple) and 40 % *Curculigopilosa*(squirrel groundnut). RHB is commercially available and the manufacturers infer that the product has the following medicinal functions amongst others, as indicated in the leaflet of the product: management/treatment of diabetes, weak erection, typhoid and malaria, vaginal discharge, menstruation anomalies, high blood pressure, waist and back pains, fibroid, infertility (male and female), gonorrhea/staphylococcus, syphilis, pile, obesity/stomach troubles, detoxifies the kidney and tones the liver, etc.

In view of these stated medicinal properties of ofRuzu herbal bitters, there is the need for scientific information on the phytochemical constituents, including the nutritional and anti-nutritional composition of the herbal mixture.

II. Materials and Methods

Materials: Ruzu herbal bitters (RHB) was purchased from the manufacturer and used directly as prepared by the manufacturer;spectrophotometer, conical flask, beaker, centrifuge, pipette, micro pipette, filter paper, measuring cylinder, electronic balance, test tube, test tube rack, funnel, stop watch, petri-dish, volumetric flasks, water bath, ethanol, methanol, ethyl acetate, hydrochloric acid, acetic anhydride, distilled water, ferric chloride, sulfuric acid, sodium hydroxide, ammonium hydroxide, sodium carbonate, etc.

Procedure Methodology

Qualitative Phytochemical Screening

Test for Alkaloids(Trease and Evans, 1989): Two milliliters (2 ml) of RHB was collected using syringe and dispensed into a test tube. The test tube was heated for 2 minutes and 5 ml of hydrogen chloride (HCL) was added and heated again and allowed to cool. The mixture was divided into two groups, A and B in two different test tubes. To A, 2 drops of Meyer's reagent was added and white precipitate was observed which showed the presence of alkaloids. To B, 2 drops of Dragendroff s reagent was added and the formation of red precipitate was observed which confirmed the presence of alkaloids.

Test for Cardiac Glycosides(Harborne, 1973): Two milliliters (2 ml) of RHB was collected into a test tube and 5 ml of glacial acetic acid was added, followed by 2 ml of $FeCl_3$ and 2 ml of concentrated ferric acid. A brown ring formation at interphase of the mixture indicated the presence of deoxy sugar characteristics of cardiac glycosides.

Test for Flavonoids(Harborne, 1973): Five milliliters (5 ml) of RHB wascollected using syringe and dispensed into a test tube. Exactly 10 ml of distilled water, 5 ml of dilute ammonium (NH_4OH) and few drops of tetraoxosulphate (IV) acid (H_2SO_4) were added in the test tube. A yellow colouration was observed which showed the presence of flavonoids.

Test for Phenols(Harborne, 1973): Exactly 5 ml of RHB was dissolved in 5 ml of distilled water. To this, few drops of neutral 5 % ferric chloride solution was added. A dark green color indicated the presence of phenolic compounds.

Test for Saponins(Harborne, 1973)

(a) Frothing test: 3 ml of the extract (RHB) was diluted with 2 ml of distilled water in a test tube. The mixture was shaken vigorously for about 5 minutes and was allowed to stand. A persistent frothing movement was observed which indicate the presence of saponins.

(b) Emulsion test: An emulsion is any thick liquid in which tiny drops of oil or fat are evenly distributed. To 3 ml of RHB was added 5 drops of olive oil in a test tube and the mixture was vigorously shaken and allowed to stand for about 30 minutes. Observation of emulsification indicated the presence of saponins.

Test for Tannins(Harborne, 1973): One milliliter (l ml) of RHB was collected using syringe and dispensed into a test tube. Then, 1 ml of (10 %) ferric chloride ($FeCl_3$) was added to the test tube. Dirty green precipitate was observed which showed the presence of tannins.

Test for Terpenoids(Harborne, 1973):A total of 2 ml of RHB was added into a test tube. To it were added 2 ml of acetic anhydride and 2 ml of concentrated H_2S0_4. A change in color from pink to violet (blue – green ring) showed the presence of terpenoids.

Test for Steroids(Trease and Evans, 1989): In 0.2 ml of RHB, 2 ml of acetic acid was added, and the solution was cooled well in ice followed by the addition of concentrated H_2SO_4 carefully. Color development from violet to blue or bluish green indicated the presence of a steroidal ring, i.e., aglycone portion of cardiac glycoside.

Quantitative PhytochemicalAnalysis

Estimation of Alkaloids(**Madhu *et al.*, 2016):**To 1ml of RHB, 5 ml pH 4.7 phosphate buffer was added and 5 ml BCG solution and the mixture shaken with 4 ml of chloroform. The extract was collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine was used as a standard material and the assay was compared with atropine equivalents.

Estimation of Glycosides Content: Into a test tube was added 1 ml of RHB and 2 ml of DNS reagent (1 g DNS dissolved in 100 ml distilled water). The test tube was put in a beaker of boiling water and boiled for 5 minutes. The test tube was cooled in cold water and 10 ml of distilled water was added. The absorbance was read at 540 nm using a spectrophotometer.

Estimation of Total Flavonoids Content (TFC) by Aluminium Chloride Method (Zhishen*et al*. (1999):To 0.1 ml of RHB, distilled water was added to make the volume up to 5 ml. To this was added 0.3 ml of 5 % $NaNO_2$ and 3 ml of 10 % $AlCl_3$, after 5 minutes later. After 6 minutes, 2 ml of 1 M NaOH was addedand the absorbance was measured at 510 nm. Rutin was used as standard for constructing a calibrationcurve.

Estimation of Total Phenolic Content (TPC) by Folin-Ciocalteu Method (Singleton and Rossi, 1965): To 0.1 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent in a test tube and incubated at room temperature for 3 minutes. Into the tube was added 2 ml of 20 % sodium carbonate and kept in a boiling water bath for 1 minute. The blue colour formed was read at 650 nm. Gallic acid was used as a standard for constructing a calibration curve.

Estimation of Saponins (Madhu *et al.*, 2016): Exactly 1 mg of RHB was dissolved in 10 ml of 80 % methanol, 2 ml of Vanilin in ethanol was added, and mixed well. Then 2 ml of 72 % sulphuric acid solution was added, mixed well and heated in a water bath at 60 ^{0}C for 10 minutes. The absorbance was measured at 544 nm against reagent blank with a spectrophotometer. Diosgenin was used as the standard absorbance curve.

Estimation of Tannins Content by modified Prussian Blue Method (Graham, 1992): To about 0.1 ml of the RHB, 6.9 ml of distilled water was added, followed by 1 ml of 0.008 M potassium ferric cyanide, 1 ml of 0.2 M ferric chloride in 0.1 M HCl, and mixed well. The blue colour formed was read at 700 nm. Tannic acid was used as a standard for constructing a calibration curve.

Estimation of Sterols by Liebermann- Burchard Method: To 1.0 ml of RHB chloroform was added to make the volume up to 5ml in a test tube. To this, 2 ml of Liberman-Burchard reagent (0.5 ml of concentrated sulphuric acid in 10 ml acetic anhydride) was added and mixed well. The green colour complex formed was measured spectrophotometrically at 640 nm. Cholesterol was used as standard for constructing a calibration curve.

Determination of Proximate Composition

Proximate analysis was carried out to determine the percentage protein, moisture, crude fibre, ash, fat and oil and carbohydrate of Ruzu herbal bitters using standard methods of analyses.

Crude Protein Determination: The crude protein content was determined as described by AOAC (2000) using microkjeldahl method. Two grams (2 g) of the sample (RHB) was weighed into Kjeldahl flask. Then, 10 ml of concentrated H_2SO_4 and 5 g of anhydrous sodium sulphate were added. It was heated in the fume chamber until solution became clear. The solution was cooled to room temperature (20 ^{0}C – 25 ^{0}C) after which it was transferred into a 200 ml volumetric flask and made up to the level with distilled water. The distillation unit was cleaned and the apparatus was set up. A 100 ml conical flask (receiving flask) containing 10 ml of 4 % boric acid and 4 drops of screened methyl red indicator was placed under the condenser. Five milliliter (5 ml) of the digest was pipetted into the apparatus through the small funnel and washed down with distilled water and followed by addition of 5 ml of 40 % NaOH solution. The distillate was collected in the receiving flask. The

distillate in the receiving flask was titrated with 0.1 N H_2SO_4 to a pink colour. The same procedure was carried out on the blank (with filter paper).

$$\% \text{ Protein} = \frac{(\text{Titre} - \text{Blank}) \; x \; 0.00014 \text{ x dilution factor x } 6.25 \text{ x } 100}{\text{Weight of sample}}$$

Moisture Content Determination: The moisture content of the sample was determined using the method of AOAC (2000). The crucible was cleaned and dried in an oven at 100 ^{0}C and then cooled in the desiccators. Two grams of sample was weighed into the crucible and dried at 100 ^{0}C in an oven until a constant weight was obtained. The percentage moisture was calculated with the expression:

$$\% \text{ Moisture} = \frac{\text{W2} - \text{W3} \; x \; 100}{\text{W2} - \text{W1}}$$

Where:
W_1 = Initial weight of empty crucible
W_2 = Weight of crucible + weight of sample before drying
W_3 = Weight of crucible + weight of sample after drying.

Determination of Ash Content: The ash content was determined using AOAC (2000) method. Two grams of the sample was weighed into a crucible and put in the preheated furnace at 550 ^{0}C until a white or light grey ash was obtained. It was cooled in a desiccator and weighed. The percentage ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{W2} - \text{W3} \; x \; 100}{\text{W2} - \text{W1}}$$

Where:
W_1 = weight of empty crucible
W_2 = weight of crucible + sample before ashing
W_3 = weight of crucible + ash

Crude Fat Content Determination: Crude fat content was determined by the Soxhlet extraction method as described by AOAC (2000). Ten grams (10 g) of sample was accurately weighed into an ashless filter paper, and placed inside an extraction thimble. The thimble was placed inside Soxhlet extractor. The extraction flask was filled with 300 ml of petroleum ether and was connected to the Soxhlet unit and then to the condenser. The heater was switched on and the unit was allowed to run for 6 hours, after which the ether was recovered before thimble was removed. The oil collected in the flask was dried at 105 ^{0}C in an oven. The extracted oil was weighed and the percentage fat was calculated as follows:

$$\% \text{ Fat} = \frac{\text{Weight of fat } x \; 100}{\text{Weight of Sample}}$$

Crude Fibre Content Determination: The method of AOAC (2000) was used. Petroleum ether (60 ^{0}C) was used to defat 2 g of the sample. This was put into 200 ml of 1.25 % H_2SO_4 and boiled for 30 minutes. The solution was filtered through muslin cloth on a fluted funnel. It was washed with boiling water until it was free of acid. The residue was put into 200 ml of 1.25 % NaOH and allowed to boil for 30 minutes. It was further washed with 1 % HCl to neutralize the NaOH and several times with hot distilled water. The final residue was drained and transferred to porcelain crucible and dried in the oven to a constant weight and cooled. The crucible and its contents were then ignited in a muffle furnace for 30 minutes at 600 ^{0}C, cooled in a desiccator and reweighed. The loss in weight was reported as percentage crude fibre.

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight after ignition } x \; 100}{\text{Weight of Sample}}$$

Determination of Carbohydrate Content: Carbohydrate content of the sample was determined by difference (AOAC, 2000).
% carbohydrate = 100 – (% Ash + % protein + % moisture + % fat + % fibre).

Determination of Mineral Contents: The mineral content of the RHB was determined using the modified method described by AOAC (2000). One gram (1 g) of the sample was digested with 100 ml of concentrated hydrochloric acid (HCl) and 20 ml of concentrated nitric acid (HNO_3) (Aqua-regia). The digest was boiled for 20 minutes at 70 ^{0}C, allowed to cool to room temperature and transferred to 50 ml volumetric flask where the volume was made up to the mark with distilled water. The digest was filtered with ashless Whatman No. 1 filter paper. The filtrate was analysed for minerals (Na, K, Mg, Mn, Ni, Pb, Cd, Fe, Zn and Cr) using flame photometry (Na and K) and atomic absorption spectrophotometer (for the heavy metals). The real values were

extrapolated from their respective standard curves. Values obtained were adjusted for Aqua-regia extractability for the respective ions.

Determination of Vitamins Content

Determination of Vitamin A: The AOAC (2000) method using colorimeter was adopted. This measures the unstable colour at the absorbance of 620 nm that results from the reaction between vitamin A and antimony trichloride ($SbCl_3$). Pyrogallol (antioxidant) was added to 2 g of sample prior to saponification with 200 ml alcoholic KOH. The saponification took place in water bath for 30 minutes. The solution was transferred to a separating funnel where water was added. The solution was extracted with 1-1.5 ml of hexane. The extract was washed with equal volume of water. The extract was filtered through filter paper containing 5 g anhydrous sodium tetraoxosulphate (VI) (Na_2SO_4) into volumetric flask. The filter paper was rinsed with hexane and made up to volume. The hexane was evaporated from the solution and blank. One milliliter (1 ml) of chloroform and antimony trichloride ($SbCl_3$) solution was added to the extract and blank. The readings of the solution and blank were taken from the colorimeter adjusted to zero absorbance.

Vitamin A (mg/mg) = A620 nm x SL x (V/Wt)

Where:

A620nm = Absorbance at 620nm
SL = Slope of standard curve (Vit A conc.) /A620 reading)
V = Final volume in colorimeter tube
Wt = Weight of sample.

Determination of Thiamin (B1) Content: The titrimetric method of AOAC (2000). Exactly 10 ml of the sample was added into a 65 ml of 0.1 N HCl in a 250 ml cornical flask. The content was heated at 60 ^{0}C in a water bath for 60 minutes with frequent shaking at a pH of about 4.5. After this time, it was then cooled and made up. About 10 ml of the extract was pipetted out into a test tube and 5 ml of alkaline potassium ferrocyanide solution added. The mixture was gently mixed together before 2 ml of concentrated H_2SO_4 was added to acidify the mixture. This was then cooled under a running tap water. Finally, 5 ml of 10 % potassium iodide solution was added. The liberated iodine was titrated against the standard thiosulphate solution.

$$\text{B1 (mg/L)} = \frac{\text{Titre value x normality of } NaS_2O_3}{\text{Volume of sample used}}$$

Determination of Riboflavin (B2) Content: Riboflavin was determined using the method of AOAC (2000). Five grams (5 g) of the sample was put in a conical flask and 50 ml of 0.2 N HCl was added to it and boiled in a water bath for an hour. It was cooled and the pH adjusted to 6.0 with NaOH. The pH was lowered by adding 1.0 N HCl, filtered into a 100 ml measuring flask and the volume was made up to mark. To remove interference, two tubes marked 1 and 2 were used. To tube 1, 10 ml of filtrate and 1 ml of water were added, while 10 ml of filtrate and 1 ml of riboflavin standard were added to tube 2. One milliliter (1 ml) of glacial acetic acid was added to each tube, mixed and then 0.5 ml of $KMnO_4$ solution was added. After 2 minutes, 0.5 ml of 3 % H_2O_2 was added and mixed well. The flourimeter was adjusted to excitation wavelength of 470 nm and emission wavelength of 525 nm. The flourimeter was adjusted to zero deflection against 0.1 N H_2SO_4 and 100 against tube No.2. The fluorescence of tube No.1 was measured. To both tubes, 20 mg of sodium sulphate was added and fluorescence measured within 10 seconds and recorded as blank reading. The riboflavin content in the sample was calculated with the expression:

$$\text{Riboflavin (mg/g sample)} = \frac{X\,x\,1}{Y - XW}$$

Where:

W = Weight of sample
X = (reading of sample 1) – (reading of blank)
Y = (reading of sample + standard tube 2) – (reading of sample + standard blank)

Determination of Niacin (B3) Content: This method was described by AOAC 1997. Five grams (5 g) of the sample was treated with 50 ml of 1 N H_2SO_4 and shaken for 1 hour. About 3 drops of ammonia solution was added to the sample and filtered. Then 10 ml of the filtrate was pipetted with 5 ml of 0.02 N H_2SO_4 and the absorbance was read using a U.V spectrophotometer at 470 nm.

$$\text{Niacin (B3)} = \frac{\text{Absorbance } x \text{ volume of cuvette}}{\text{Volume of sample x } 1000}$$

Determination of Ascorbic Acid (C) Content: The vitamin C content of the samples was determined using 2, 4-dinitrophenyl hydrazine (DNPH) method as described by AOAC (2000). The sample (20 g) was extracted

(blended) with 200 ml of 6% trichloroacetic acid (TCA) solution and filtered. Small quantity of animal charcoal was added to the filtrate to decolorize it. One millilitre (1ml) of the filtrate was pipetted into a test tube. Ascorbic acid standard (1 ml) was also pipetted into another test tube and 1ml of 6% trichloroacetic acid was pipetted into a third tube as a blank. Dinitrophenyl hydrazine-theourea-copper sulphate (1 ml) reagent (DTCS) was added to all the test tubes, capped, mixed and incubated in a water bath at 37 ^{0}C for 3 hours. The tubes were removed from the water bath and chilled for 30 minutes in an ice bath while mixing slowly. Two millilitres (2 ml) of 12 M sulphuric acid was added to all the test tubes. The spectrophotometer was adjusted with the blank to read zero absorbance at 520 nm. The absorbances of the standard and test samples were then read. The determination was carried out in triplicates. The ascorbic acid content of the sample was calculated with the expression:

$$\text{Ascorbic acid (mg)} = \frac{\text{Absorbance of test sample } x \text{ Concentration of Standard}}{\text{Absorbance of standard x Weight of the sample}}$$

Determination of vitamins D and K: A method as described by AOAC (2005) was used. Into a 250 ml cornical flask containing 100ml of petroleum ether was added 10 g of the sample and shaken with an adjustable vibrator shaker for 3 hours at room temperature. After shaking, it was allowed to stand for 10 minutes. The mixture was carefully decanted into a 500 ml separating funnel and equal volume of water was added to remove traces of impurities. The supernatant was carefully collected and the absorbance was read for both vitamin D and K at 250 nm and 248 nm respectively.

Determination of Vitamin E Content: The method of Pearson (1976) was used. One gram of the sample was weighed into a 100 ml flask where 10 ml of absolute alcohol and 20 ml of alcoholic tetraoxosulphate (VI) acid (H_2SO_4) were added. Ten milliliters (10 ml) of the clear solution was pipetted into a test tube, heated in a water bath at 90 ^{0}C for 30 minutes and allowed to cool. The absorbance was read in a spectrophotometer at 470 nm wavelength. The blank and the standard vitamin E solutions were prepared and vitamin E was calculated with the expression:

$$\text{Vitamin E (mg/100 g)} = \frac{\text{a} - \text{b } x \text{ } c}{\text{s} - \text{bw}}$$

Where:

a = Absorbance of test sample
b = Absorbance of the standard solution
c = Concentration of standard in mg/100g
w = Weight of the sample used.

Determination of Amino Acid Profile

The Amino Acid profile of the known sample was determined using the method described by Benitez (1989). The RHB sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Bio-systems PTH Amino Acid Analyzer.

Determination of Antinutrients

Determination of Tannin Content: The Folin-Denis colorimetric method as described by Kirk and Sawyer (1998) was used to determine tannin content in the sample. The sample (5 g) was dispersed in 50 ml of distilled water and agitated. The mixture was allowed to stand for 30 minutes at roomtemperature before it was filtered through what man No.42 grade of filter paper. The extract(2 ml) was taken into a 50 ml volumetric flask. Similarly, 2 ml tannin solution (tannic acid) and2 ml of distilled water were put in separate 50 ml volumetric flasks to serve as standard andreagent blank respectively. Then 1.0 ml of Folin-Denis was added to each of the flask, followed by addition of 2.5 mlof saturated sodium carbonate solution. The content of each flask was made up to 50 ml withdistilled water and allowed to incubate for 90 minutes at room temperature. Their respectiveabsorbances were measured in a spectrophotometer at260 nm using the reagent blank to calibrate the instrument at zero. The tannin content wascalculated using the formula:

$$\% \text{ Tannins} = \frac{\text{An } \times \text{ C } \times \text{ Vf } \times 100}{\text{AS x W x Va}}$$

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

Determination of Alkaloid Content: This was done by alkaline precipitation gravimetric method as described by Harbourne (1973). A known weight (5 g) of the sample was dispersed in 10 % acetic acid solution in ethanol to form a ratio of 1:10 (10 %). The mixture was allowed to stand for 4 hours at 28 ^{0}C. It was later filtered through Whatman No. 1. grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous ammonia (NH_4OH) until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1 % ammonia solution and dried in the oven at 80 ^{0}C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

Determination of Phytate Content: The method described by Oberleas (1973) was used for the determination of phytic acid. Two grams (2 g) of the sample was weighed into a 100 ml flask and extracted with 50 ml of 0.2 N HCl. Five millilitres (5 ml) of the extract was measured out into a test tube fitted with a glass stopper. One millilitre (1 ml) of solution prepared by dissolving 0.2 g of ammonium iron (III) sulphate in 2N HCl and made up to 100 ml with distilled water was added to the extract. The tube was heated in a boiling water bath for 30 minutes and cooled in ice for 15 minutes before it was adjusted to room temperature. The content of the tube was mixed and centrifuged for 30 minutes at 3000 rpm. One millilitre (1 ml) of the supernatant was transferred to another test tube and 1.5 ml of solution made by dissolving 10 g 2, 2- bipyridine and 100 ml thioglycollic acid in distilled water and made up to 1000 ml was added to it. The absorbance was measured at 519 nm against distilled water. Calibration curve was prepared by plotting the concentration of the reference solution (phytate reference solution) against their corresponding absorbance. The absorbance of the test sample was then used to obtain the concentration from the calibration curve.

Determination of Oxalate Content: This was determined by the method of Dye (1956) with slight modification as described by Iwuoha and Kalu (1995). Two grams of the sample was suspended in a mixture of 190 ml of distilled water and 10 ml of 6 N HCl in a 250 ml volumetric flask, digested for 1 hour at 100 ^{0}C, cooled and made up to 250 ml with distilled water. The digest was filtered through Whatman No 1 filter paper using a suction pump. A duplicate portion of 125 ml of the filtrate was measured into 250 ml beaker and 4 drops of methyl red indicator added to each beaker. Concentrated NH_3 solution was added drop wise until the test solution changed from its salmon pink colour to a faint yellow colour (pH 4 - 4.5). Each portion was heated to 90 ^{0}C and 10 ml of 5 % $CaCl_2$ was added while stirring constantly. After heating, it was cooled and left overnight at 5 ^{0}C. The solution was centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and the precipitate was completely distilled in 10 ml of 20 % H_2SO_4 solution. At this point, the filtrate resulting from digestion of 2 g of the sample was combined and made up to 300 ml. Aliquots (125 ml) of the filtrate was heated until near – boiling and then titrated against 0.05 M standard $KMNO_4$ solution to a faint pink colour which persisted for 30 seconds. Oxalic acid content was calculated using the formula:

$$\%\ \text{Oxalic acid} = \frac{\text{T x (Vme) (DF) x 105}}{\text{ME x MF}}$$

Where;
T = Titre of $KMNO_4$ (ml)
Vme = Volume – mass equivalent to 0.002 g anhydrous oxalic acid.
DF = Dilution factor (i.e. 300 ml/125 ml)
ME = Molar equivalent of $KMnO_4$ in oxalic acid ($KMnO_4$ redox reaction is 5)
MF = Mass of sample used.

Determination of Cyanogenic Glycoside: The alkaline titration method of AOAC (2000) was used for the determination of cyanogenic glycoside in the sample. Distilled water (200 ml) was added to 1.0 g of sample (in triplicate) in 800 cm^3 capacity distillation flask. Silicon oil was also added as an antifoaming agent. The flask was fitted for distillation and allowed to stand for 2 hours for autolysis to take place. Steam distillation was carried out and 150 ml of the distillate collected into a 250 ml capacity conical flask containing 20 ml of 2.5 % sodium hydroxide and diluted to mark with distilled water. To 100 ml of diluted distillate containing the cyanogenic glycoside, 8.0 ml of 6 N sodium hydroxide solution and 2.0 ml of 5 % potassium iodide were added. This was titrated against 0.02 N silver nitrate ($AgNO_3$) solution using a 10ml micro burette. The end-point was noted as a permanent turbidity against a black background. The HCN content was then calculated with the expression:

$$\text{HCN (mg)} = \frac{1.08 \times \text{Titre} \times \text{Dilution factor} \times 100}{\text{Weight of sample}}$$

Determination of Cardiac Glycoside: One gram (1 g) of the extract was extracted with 10 ml 70 % alcohol and the mixture filtered. From the filtrate, 8 ml was transferred to a 100 ml volumetric flask and the volume was

completed to the mark with distilled water. Then 8 ml of the mixture was added to 8 ml of 12.5 % lead acetate (to precipitate resins, tannins and pigments). The mixture was shaken well, completed to the volume of 100 ml with distilled water and filtered. Exactly 50 ml of the filtrate was pipette into another 100 ml volumetric flask and 8 ml of 4.7 % disodium hydrogen phosphate (Na_2HPO_4) solution was added to precipitate excess lead. The mixture was made up to the volume with distilled water and mixed. This was filtered twice through filter paper. Baljets reagent (10 ml) was added to 10 ml of the purified filtrate. A blank sample of 10 ml of distilled water was also added to 10 ml Baljets reagent. The two solutions were allowed to stand for one hour (time necessary for maximum colour development) a blank of 20 ml distilled water was used. The intensity of the colour was read at 495 nm using spectrophotometer. The colour was stable for several hours (Trease and Evans, 1989).

$$\% \text{ Glycosides} = \frac{A \times 100 \text{ g } \%}{17}$$

Where A = the absorbance of the colour at 495nm.

Estimation of Saponin Content: A total of 1 mg of the extract was dissolved in 10 ml of 80 % methanol; 2ml of vanilin in ethanol was added and mixed well. Also, 2 ml of 72 % sulphuric acid solution was added, mixed well and heated on a water bath at 60 ^{0}C for 10 minutes. The absorbance of the mixture was measured at 544 nm against reagent blank. Diosgenin was used as the standard curve.

Statistical Analysis

The data obtained was analyzed by one-way ANOVA using the SPSS Statistical package version 20.0. All data was expressed as mean ± SD of triplicates (n = 3) and hypothesis was tested at 95% level of significance.

III. RESULTS

Qualitative Phytochemical Analyses

Table no 1 shows the qualitative phytochemical analysis of Ruzu herbal bitters. The result showed that RHB contains alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins and steroids, while terpenoids were absent.

Table no 1. Qualitative phytochemical analysis of Ruzu herbal bitters

Phytochem1cals	**Result**
Alkaloids	+
Cardiac glycosides	+
Flavonoids	+
Phenols	+
Saponins	+
Tannins	+
Steroids	+
Terpenoids	-

Key: + = Present, - = Absent

Quantitative Phytochemical Analyses

Table no 2shows the quantitative phytochemical analysis of Ruzu herbal bitters. The result revealed that the product is rich in saponins (0.77 mg/ml), alkaloids (0.76 mg/ml), flavonoids (0.73 mM), and cardiac glycosides (0.32 mM); phenols (0.09 mg/ml) and steroids (0.09 mg/ml) are moderately present while tannins (0.01) are least amongst the phytochemicals analyzed.

Table no 2. Quantitative phytochemical analysis of Ruzu herbal bitters

Phytochem1cals	**Concentration**
Alkaloids (mg/ml)	0.76 ±0.00
Saponins (mg/ml)	0.77 ± 0.01
Flavonoids (mM)	0.73 ± 0.02
Tannins (mg/ml)	0.01 ± 0.00
Phenols (mg/ml)	0.09 ± 0.00
Steroids (mg/ml)	0.09 ± 0.00
Cardiac glycosides (mM)	0.32 ± 0.01

Valuesare of three replicates ± standard deviations

Proximate Composition

Table no 3 shows the proximate composition of Ruzu herbal bitters. The result showed that the product has a high ash content (57.2 %) and reasonable amounts of moisture (14.16 %), carbohydrate (12.35 %), fibre (11.13 %) and protein (4.87 %). with low percentage of fat (0.60 %).

Table no 3: Result of proximate analysis of Ruzu herbal bitters

Parameters	% Composition
Moisture	14.16 ± 0.01
Ash	57.2 ± 0.01
Crude fibre	11.13 ± 0.01
Crude fat	0.60 ± 0.05
Protein	4.87 ± 0.01
Carbohydrate	12.35 ± 0.00

Values are mean of three replicates ± standard deviations

Mineral Composition

Table no 4 shows the mineral composition of Ruzu herbal bitters. The result showed that the product contains the following minerals in a decreasing order of amount: magnesium (1.84 mg/l), lead (0.35 mg/l), potassium (0.30 mg/l), sodium (0.10 mg/l), iron (0.08 mg/l), chromium (0.03 mg/l), cadmium (0.01 mg/l) and manganese (0.01 mg/l).

Table no 4: Mineral composition of Ruzu herbal bitters

Minerals	Concentration(mg/1)
Magnesium (Mg)	1.84 ± 0.02
Potassium (K)	0.30 ± 0.01
Sodium (Na)	0.10 ± 0.01
Manganese (Mn)	0.01 ± 0.00
Lead (Pb)	0.35 ± 0.00
Iron (Fe)	0.08 ± 0.00
Chromium (Cr)	0.03 ± 0.00
Cadmium (Cd)	0.01 ± 0.00

Values are mean of three replicates ± standard deviations

Vitamins Content

Table no 5 shows the vitamins content of Ruzu herbal bitters is presented in Table 5. The result showed that the product contains relative amounts of both water and fat soluble vitamins with relatively higher amounts observed in vitamins K (1.67 mg/1), D (1.37 mg/1), C (1.35 mg/1), A (1.04 mg/1), B3 (0.94 mg/1) and B2 (0.71 mg/1), while lower amounts were observed in vitamins E (0.030 mg/1) and B1 (0.024 mg/1).

Table no 5: Vitamins contained in Ruzu herbal bitters

Vitamins	Concentration(mg/1)
Vitamin A	1.04 ± 0.00
Vitamin B1	0.02 ± 0.00
Vitamin B2	0.71 ± 0.01
Vitamin B3	0.94 ± 0.00
Vitamin C	1.35 ± 0.00
Vitamin D	1.37 ± 0.00
Vitamin E	0.03 ± 0.00
Vitamin K	1.67 ± 0.00

Values are mean of three replicates ± standard deviations.

Amino Acid Profile

Table no 6 shows the amino acid profile of Ruzu herbal bitters, whichindicated the presence of 18 amino acids. Glutamic acid (5.15 g/100g) has the highest concentration, followed by leucine (3.56 g/100g) and aspartic acid (3.44 g/100g) while tryptophan (0.26) has the lowest concentration, followed by cysteine (0.48 g/100g) and methionine (0.53 g/100g). Glutamine and asparagine are absent.

Table no 6: Amino Acid Profile of Ruzu herbal bitters in g/100 g protein

Amino Acids	Concentration (g/100g)
*Leucine	3.56
*Lysine	2.17
*Isoleucine	2.23
*Phenylalanine	3.02
*Tryptophan	0.26
*Valine	1.81
*Methionine	0.53
Proline	0.61
*Arginine	2.06
Tyrosine	1.03
*Histidine	0.80
Cysteine	0.48
Alanine	2.28
Glutamic Acid	5.15
Glycine	0.90
*Threonine	1.00
Serine	0.84
Aspartic Acid	3.44

* = Essential Amino acid

Antinutritional Constituents

Table no 7 showsthe antinutritional constituents of Ruzu herbal bitters. The result showed that the product has the highest percentage composition of saponins (76.73 %) and cardiac glycosides (36.13 %). Others are phytates (1.33 %), oxalates (1.15 %), alkaloids (0.93 %), cyanogenic glycosides (0.80 %) and tannins (0.01 %).

Table 7: Result of anti-nutritional analysis of Ruzu herbal bitters

Antinutrients	% Composition
Tannins	0.01 ± 0.00
Cyanogenic Glycosides	0.80 ± 0.01
Saponins	76.73 ± 0.58
Alkaloids	0.93 ± 0.00
Oxalates	1.15 ± 0.00
Cardiac Glycosides	36.13 ± 57.80
Phytates	1.33 ± 0.00

Analyses were mean of three replicates ± standard deviations

III. Discussion

Phytochemical Analysis

The qualitative phytochemical analysis of Ruzu herbal bitters (RHB) in Table 1 revealed the presence of medicinally active secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides and sterols. The result of the quantitative phytochemical analysis of RHB presented in Table 2 showed that the product has high concentrations of saponins (0.77 mg/ml), alkaloids (0.76 mg/ml), flavonoids (0.73 mM), and cardiac glycosides (0.32 mM); whereas the concentrations of phenols (0.09 mg/ml), steroids (0.09 mg/ml) and tannins (0.01 mg/ml) are low amongst the phytochemicals analyzed. Phytochemicals have been reported to have different levels of pharmacological activities such as anti-inflammatory, antiatherosclerotic, antituumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Badam*et al.*, 2002; Sala *et al.*, 2002; Gupta and Tandon, 2004).

The presence of alkaloids in Ruzu herbal bitters makes it recommendable for treatment of ailments, since alkaloids possess significant pharmacological and physiological activities when administered to animals (Aborisade, 2017). Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic, antispasmodic and have bactericidal effects (Stray, 1998). Flavonoids have different biochemical and pharmacological functions such as antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic, activities (Middleton and Andkandaswami, 1993). Flavonoids in intestinal tract lower the risk of heart disease (Okwu, 2005). Phenols and phenolic compounds are extensively used in disinfection and remain the standard with which other bactericides are compared (Osuagwu*et al.*, 2007). Tannins have been reported to possess astringent properties, hasten the healing of wound and inflamed mucous membranes (Okwu, 2004). Furthermore, tannins have soothing relief,

help to regenerate the skin, have anti- inflammatory and diuretic effects (Okwu and Okwu, 2004). Tannins from the part of the plant bark, root, stem and other part of many plants especially *Euphorbiaceae* are used to treat cells that have gone neoplastic (Duke and Wain, 1981). This suggests that tannins are also used in the treatment of wounds emanating from varicose ulcer and haemorrhoids (Nguyi, 1988; Njoku and Akumufula, 2007) and to stop bleeding during circumcision (Joshua, 2006).

Saponins are usually detected by their ability to foam persistently and to haemolyze blood cells (Trease and Evans, 1989). They have been of medicinal value for centuries due to their common biological property of cytotoxicity (Nobori*et al.*, 1994). Saponins are known to have inhibitory effect on inflammation (Just *et al.*, 1998). They have the property of precipitating and coagulating red blood cells (Uma and Sekar, 2014). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo*et al.*, 2000;Okwu, 2004). Steroids have been reported as the main treatment of inflammatory conditions, such as systemic vasculitis (inflammation of blood vessels) and myositis (inflammation of muscles); and are important component of cell membrane which alter membrane fluidity, whilesomefunction as signaling molecules which activate steroid hormone receptors (Rhen and Cidlowski, 2005). Sterols help in the lowering of plasma cholesterol and low-density lipoprotein cholesterol. Hence, its inclusion in the ruminant diet will assist in reducing drastically the morbidity and mortally caused by cardiovascular disease (Uma and Sekar, 2014).Glycosides have their clinical effect in cases of congestive heart failure to increase the force of myocardial contraction. They exert their hypotensive effect by inhibiting Na^+-K^+ ATPase (Brian *et al.*, 1985).Therefore, the presence of the various phytochemicals in Ruzu herbal bitters is an indication that the polyherbal mixture would be medicinally efficacious in the treatment of several ailments.

Nutritional Constituents

The result of the proximate composition in Table 3 showed that RHB is a rich source of energy with carbohydrate content of 12.35 %. Carbohydrates are the primary sources of energy for the maintenance and sustenance of health and life in animals, including man. The product has low concentration of crude fat at 0.60 %, having the lowest concentration amongst the nutrients analysed. Fat is an alternative source of energy during fasting, but excess fat deposits can lead to cardiovascular disorders and obesity, a predisposing factor to diabetes mellitus. The low level of fat in RHB could be responsible for its antihyperlipidemic activity. The crude protein concentration showed a percentage value of 4.87, which is a nutritional advantage. Proteins are essential in the formation of hormones, enzymes, neurotransmitters, biological amines, and control of a variety of body functions such as growth, repair and maintenance of body protein (Pazhanichamy*et al*., 2009). Proteins are also useful in gluconeogenesis during starvation as alternative source of energy, from glucogenic amino acids.

Crude fibre was found to be at 11.13 %. The Crude fibre content is recognized as a useful ingredient for the control of oxidative processes in food products and as a functional food ingredient (Mandalari*et al*., 2010).Crude fibreaffects the absorption of cholesterol from the gut. It also delays digestion and conversion of starch to sugars, an important factor in the management of diabetes. Diet high in fibre may also protect against cardiovascular diseases, colorectal cancer and obesity (Cust *et al*., 2009). Fibre is equally useful against constipation. Moisture content was high at 14.16 %. The product showed highest concentration of ash content at 57.20 %. High ash content is an indication that RHB contains much minerals necessary for normal physiological function.

The result of mineral content of RHB presented in Table 4 showed that the product contains magnesium (Mg) in the highest amount and lower amounts of potassium (K), iron (Fe) and sodium (Na); whereas chromium (Cr), cadmium (Cd), lead (Pb) and manganese (Mn) are insignificantly present. This indicates that Ruzu herbal bitters could be a good source of minerals/trace elements. Magnesium is important in carbohydrate metabolism as it serves as a cofactor in glycolysis. Macronutrients compositions especially sodium and potassium are essential cations abundantly present in plants. The concentrations of Pb, Cr, Cd and Mn in RHB (0.01 mg/L each) are lower than the recommended level for toxicity in plants (Hussain *et al*., 2009).

RHB contains relative amounts of both water- and fat-soluble vitamins (Table 5). The result showed relatively higher amounts of vitamins K (1.67 mg/1), D (1.37 mg/1), C (1.35 mg/1), A (1.04 mg/1), B3 (0.94 mg/1) and B2 (0.71 mg/1), while lower amounts were observed in vitamins E (0.030 mg/1) and B1 (0.024 mg/1). This showed that RHB is rich in vitamins.In line with their nutritional importance, vitamins B1, B2 and B3 are precursors of thiamine pyrophosphate (TPP), flavin coenzymes (FMN, FAD) and nicotinamide coenzymes (NAD^+, $NADP^+$) respectively, which are necessary for the activity of enzymes involved in various biochemical reactions. Vitamin C (ascorbic acid) concentration of 1.35 mg/l indicates that RHB is high in ascorbic acid which is an antioxidant and free radical scavenger. Vitamin D (Cholecalciferol) was also observed to be high in RHB.Apart from the skin protection and mineralization of bones, the presence of vitamin D in the body increases absorption of nutrient from the small intestine especially calcium and phosphorus (regulates calcium/phosphorus plasma levels), also enhances increased renal excretion of phosphorus;which in turn reduces the action of antinutrients (oxalate) in the body (Norman, 1990).

The result of the amino acid profile of Ruzu herbal bitters in Table 6 indicated the presence of 18 amino acids. Glutamic acid (5.15 g/100g) has the highest concentration, followed by leucine (3.56 g/100g) and aspartic acid (3.44 g/100g) while tryptophan (0.26) has the lowest concentration, followed by cysteine (0.48 g/100g) and methionine (0.53 g/100g). Glutamine and asparagine are absent. These two amino acids could be lost during acid hydrolysis in which they are converted to glutamic acid and aspartic acid, respectively. The amino acids produced in plant systems act as osmolyte, regulate ion transport, modulate stomata opening, activate phytohormones and growth substances, generate chelating effect on micronutrients and play a vital role in the detoxification of heavy metals. They are also responsible for the synthesis and functional properties of specific enzymes, gene expression, and redox-homeostasis (Zhao *et al.*, 1998). Most importantly, in higher plants the amino acids serve as precursors for secondary metabolism (Zhao *et al.*, 1998). The nutritional composition of RHB is suggestive that the product will add to the bulk of nutrients in maintaining normal growth and development of the body.

Antinutritional Constituents

Antinutrients result showed the presence of tannins, cardiac glycosides, alkaloids, oxalates, cyanogenic glycosides, phytates and saponins (Table 7). The presence of these secondary metabolites could contribute to the medicinal value of RHB. Tannins are complex phenolic polymers which can bind to proteins and carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Nwogu *et al*., 2008). Oxalates function as chelating agents and may chelate many toxic metals such as mercury and lead. One major concern or difference between oxalate and other chelating agents is that oxalates could trap heavy metals in the tissues of living organism thereby making their elimination very difficult (Egbuna and Ifemeje, 2015). In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in the urine as minute crystals. Oxalate crystals can be razor sharp and may cause damage to various tissues. The sharp crystals cause damage due to their physical structure, but any contact with the crystals also increases inflammation. Iron oxalate crystals cause significant oxidative damage and diminish iron stores needed for red blood cell formation whereas many kidney stones result from calcium crystals (Egbuna and Ifemeje, 2015). Hence, calcium oxalate crystals play a role to the formation of kidney stones in the urinary tract when the acid is excreted in the urine (Nachbar*et al*., 2000). However, at moderate levels, as found in our study, the antinutrients help to regulate the availability of the nutrients. Therefore, RHB is good for consumption based on the levels of the various antinutrients present in it.

IV. Conclusion

Ruzu herbal bitters, a polyherbal mixture produced in Nigeria used in the treatment of several diseases have been found, in our study, to contain different phytochemicals that have several medicinal values. The presence of these phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides and sterols in the product are suggestive of its medicinal usefulness in the treatment of various ailments. It is also rich in different nutrients,which could add to the bulk of body nutrients; and antinutrients, which influence nutrients' availability; thereby enhancing physiological activities.

Recommendation

We recommend that further studies be carried out with animals to ascertain the biochemical effects of Ruzu herbal bitters in animals.

Appreciation

We wish to appreciate the management of Ruzu Natural Health Product and Services, Nigeria, for permitting us to use their product for research.

Conflict of Interest: None.

References

[1]. Aborisade, A. B., Adetutu, A. and Owoade, A. O. (2017). Phytochemical and Proximate Analysis of Some Medicinal Leaves. *Clinical Medicine Research*, **6** (6): 209 – 214.

[2]. Abugassa, I. O., Bashir, A. T., Doubali, K., Ekvir, R. H., Abu-Enavel, M. and Abugassa, S. O. (2008). Characterization of Trace Elements in Medicinal Herbs by Instrumental Neuron Activation Analysis. *Journal of Radioanalogy and Nuclear Chemistry*, **278** (3): 559 - 563.

[3]. Anderson, G. D. (2004). Phytochemical. Dynamic Chiropractic, In K. T. Chung, I. Weichang and M. G. Johnson. Are tannins a double edge sword in biology and health? *Trends in Food Science Technology*,**4:** 168 - 175.

[4]. AOAC (2005) Official Methods of Analysis of Association of Official Analytical Chemists. 18th Edition, Washington, DC. Washington DC, Method 935.14 and 992.24.

[5]. AOAC. (1997). Official Methods of Analysis of AOAC International, 16th edition, Association of Official Analytical Chemists, Washington, DC.

[6]. AOAC. (2000). Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24.

[7]. Badam, L., Bedekar, S. S., Sonawane, K. B. and Joshi, S. P. (2002). *In vitro* Antiviral Activity of Bael (*Aegle marmelos* Corr.) Upon Human Coxsackieviruses B1-B6. *Journal of Communicable Diseases*,**34** (2): 88 – 99.

[8]. Benitez, L. V. (1989). Amino acid and fatty acid profiles in aquaculture nutrition studies, In: S.S. De Silva (ed.), Fish Nutrition Research in Asia, Proceedings of the Third Asian Fish Nutrition Network Meeting, Manila, Philippines: Asian Fisheries Society. Pp. 23 - 35.

[9]. Brian, F. H., Thomas-Bigger, Jr. J and Goodman, G. (1985). The pharmacological basis of therapeutics, 7th Edition, Macmillan Publishing Company, NY, pp. 716 - 718.

[10]. Cai, Y. Z. Sun, M. and Corke H. (2003). Antioxidant activity of betalains from plants of the *Amaramhaceae.Journal of Agriculture and food chemistry;*

[11]. Cust, A. E., Skilton, M. R. and Van Bakel, M. M. E. (2009). Total dietary carbohydrate, sugar, starch and fibre intakes in the European Prospective Investigation into cancer and nutrition. *EuropeanJournal of Clinical Nutrition*, **63**: 37 – 60.

[12]. Del-Rio, A., Obdululio, B.C., Casflllo, J., Main, F.G., Ortuno, A. (1997). Uses and properties of citrus flavonoids. *Journal of Agriculture, Food and Chemistry*, 45:4505-4515.

[13]. Duke, J. A. and Wain, K. K. (1981). Medicinal plants of the world. Computer Index with More than 85,000 entries, Volume 3, Plants Genetics and Germplasm Institute, Agriculture Research Service, Beltsville, Maryland.

[14]. Dye, V. B. (1956). Chemical studies on *Halogetonglumeratus.Weeds*,**4:** 55 - 60.

[15]. Egbuna, C. and Ifemeje, J. C. (2015). Biological Functions and Anti-nutritional Effects of Phytochemicals in Living System. *IOSR Journal of Pharmacy and Biological Sciences*,**10** (2): 10 – 19.

[16]. Graham, H. D. (1992). Stabilization of the Prussian blue color in thedetermination of polyphenols. *Journal of Agriculture, Food andChemistry*, **40**(5):801 - 805.

[17]. Güçlü-Ustündağ, O. andMazza, G. (2007). Saponins: properties, applications and processing. *Critical ReviewsinFood Scienceand Nutrition*,**47:** 231 – 258.

[18]. Gupta, A. K. and Tandon, N. (2004). Reviews on Indian Medicinal Plants. Vol. 1. Indian Council of Medicinal Research, New Delhi, India. p. 543.

[19]. Harborne, J. B. (1973). Phytochemicals Methods. Chapman and Hall Ltd., London. Pp. 49 - 188.

[20]. Hussain, J., Khan, A. L., Rehman, N., Hamayun, M., Shah, T., Nisar, M., Bano, T., Shinwari, Z. K. and Lee, I. J. (2009). Proximate and nutrient analysis of selected vegetable species: A case study of Karak region, Pakistan. *African Journal of Biotechnology*, **8**(12):2725-2729.

[21]. Ifemeje, J. C., Egbuna, C., Eziokwudiaso, J. O. and Ezebuo, F.C. (2014). Determination of the Anti-nutrient Composition of *Ocimumgratissimum, Corchorus olitorius, MurrayakoenigiiSpreng*and *Cucurbita maxima. International Journal of Innovation and Scientific Research*,**3** (2): 127 – 133.

[22]. Ihekoronye, A. I. and Ngoddy, P. O. (1985). Integrated Food Science and Technology. Macmilian Publishers, New York. Pp. 296 - 301.

[23]. Iwuoha, C. I. and Kalu, F. A. (1995). Calcium oxalate and physico-chemical properties of cocoyam (*Colocasia esculents* and *Xanthosomasaqiltifolnim*) tuber flours as affected by processing. *Food Chemistry*,**54:** 61 - 66.

[24]. Joshua, K. (2006). Conservation of Indigenous Medicinal botanicals in Ekiti State, Nigeria. *JournalofZheijangUniversity SCIENCE B*,**7:** 713 - 718.

[25]. Just, M. J., Recio, M. C., Giner, R. M., Cucller, M. U., Manez, S. and Billia, A. R. (1998). Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticescens,* **64:** 404 - 407.

[26]. Kirk, H. and Sawyer, R. (1998). Frait Pearson Chemical Analysis of Food. 8th edition. Longman Scientific and Technical. Edinburgh. Pp. 211 - 212.

[27]. Kitts, D. D., Yuan, Y. V., Wijewickreme, A. N. and Hu, C. (2000). Antioxidant Properties of a North American gingseng extract. *Molecular CellBiochemistry*, **48:** 5672 - 5676.

[28]. Liebermann, C. (1885). Ueber das Oxychinoterpen.*ChemischeBerichte*,**18**: 1803 – 1809

[29]. Madhu, M., Sailaya, V., Satyadev, T. N. and Satyanarayana, M. V. (2016).Quantitative Phytochemical analysis of selected medicinal plant speciesby using various organic solvent. *Journal of Pharmacognosy andphytochemistry*, **5** (2): 25 - 29.

[30]. Mandalari, G., Tomaino, A., Arcoraci, T., Martorana, M.,Turco, V. L., Cacciola, F., Rich, G. T., Bisignano, C., Saija, A., Dugo, P., Cross, K. L., Parker, M. L., Waldron, K. W. and Wickham, M. S. J. (2010). Characterization of polyphenols, lipids and dietary fibre from almond skins (*Amygdalus communis*L.). *Journal of Food Composition and Analyses*, **23** (2): 166 - 174.

[31]. Middleton, E. and Andkandaswami, C. (1993). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer, in the flavonoids, advances in research science (Wd.) Horborn, I. R., Chapman and hall, London. Pp. 619 - 645.

[32]. Muselik, J., Garcia-Alonso, M., Martin-Lopez, M. P., Zelmieka, M. and Rivas-Gonzalo, J. C. (2007). Measurement of Antioxidant Activity of Wine Catechins, Procyanidins, Antocyanins and Piranoantocyanins. *International Journal of molecular Science*, **8:** 797 - 809.

[33]. Nachbar, M. S., Oppenheim, J. D. and Thomas, J. O. (2000). Lectins in the US diet: Isolation and characterization of a lectin from the tomato (Lycopersicon). *Journal of Biological Chemistry*, **255:** 2056 - 2063.

[34]. Nguyi, A. A. (1988). Tannins of some Nigerian flora. Niger. *Journal of Biotechnology*,**6:** 221 - 226.

[35]. Njoku, P. C. and Akumufula, M. I. (2007). Phytochemical and nutrient evaluation of spondiasmombi Leave. *Pakistan Journal of Nutrition*, **6** (6): 613 - 615.

[36]. Nobori, T., Miurak, K., Wu, D. J., Takaboyashik, L. A. and Carson, D. A. (1994). Detection of cyclin dependent, Kinase-4 inhibition gene in multiple human cancers. *Nature*, **46:** 753 - 756.

[37]. Norman, A. W. (1990). Intestinal calcium absorption: a vitamin D-hormone mediated adaptive response. *American Journal of Clinical Nutrition*,**51**: 290 – 300.

[38]. Nwogu, L. A., Igwe, C. U. and Emejulu, A. A. (2008). Effects of *Landolphiaowariensis* leaf extract on the liver function profile and haemoglobin concentration of albino rats. *African Journal Biotechnology*, **2** (12): 240 - 242.

[39]. Oberleas, D., (1973). Phytates. In: Toxicants Occurring Naturally in Foods, Strong, F. (Ed.). National Academic of Sciences, Washington, DC. Pp. 363 - 371.

[40]. Okwu, D. E. (2004). Phytochemicals and vitamin content of indigenous species of Southeastern Nigeria. *Journal of Agriculture and Environment*, **6** (1): 30 - 37.

[41]. Okwu, D. E. (2005). Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants. *International Journal of Molecular Medicine and Advance Sciences*, **1** (4): 375 – 381.

[42]. Okwu, D. E. and Okwu, M. E. (2004). Chemical composition of *Spondiasmombinlinn*, plant parts.*Journal of Sustainable Agriculture and the Environment*, **6** (2): 140 - 147.
[43]. Osuagwu, G. G. E., I.C. Okwulehie, I. C. and Emenike, J. O. (2007). Phytochemical and mineral content of the leaves of four Nigerian *Pterocarpus* (JACQ) species. *International Journal* of *Molecular Medicineand Advance Sciences*, **3:** 6 - 11.
[44]. Pazhanichamy, K., Pavithra, S., Rubini, S., Lavanya, B., Ramya, I. and Eevera, T. (2010). Morphological, anatomical and proximate analysis of leaf, root, rhizome of *CostusIgneus*. *Journal of Pharmacological Research*, **3** (4): 747 - 752.
[45]. Pearson, D. A. (1976). Chemical Analysis of Foods. 7th Edition. Church Hill Livingstone, New York. Volume 1. Pp. 422 - 511.
[46]. Rajurkar, N. S. and Damame, M. M. (1998). Mineral content of medicinal plants used in treatment of diseases resulting from urinary tract disorders. *Applied Radiation and Isotopes*, **49** (7): 773 - 776.
[47]. Rhen, T. and Cidlowski, J. A. (2005). Anti-inflammatory action of glucocorticoids - new mechanisms for old drugs. *New England Journal of Medicine*, 353: 1711 – 1723.
[48]. Saidulu, C. H., Venkateshwar, C. and Gangadhar, R. S. (2014). Preliminary phytochemical studies of medicinal plant drug: withaniasomniferalinn. *International quarterly journal of biology & life sciences*,**2** (1): 306 - 312.
[49]. Sala, A., Recio, M. D., Giner, R. M., Manez, S., Tournier, H., Schinella, G. and Rios, J. L. (2002). Anti-inflammatory and antioxidant properties of *Helichrysumitalium. Journal ofPharmacognosy pharmacology*, **54** (3): 365 - 371.
[50]. San Martín, R. and Briones, R. (1999). Industrial uses and sustainable supply of *Quillajasaponaria* (Rosaceae) saponins. *Economic Botany*,**53:** 302 – 311
[51]. Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*,**16:** 144 - 153.
[52]. Sodipo, O. A., Akiniyi, J. A. and Ogunbomosu, J. U. (2000). Studies on certain on certain characteristics of extracts of bark of *Pausinystaliamacroceras*(K Schemp) PicrreExbeille. Global *Journal of Pure and Applied Science*,**6:** 83 - 87.
[53]. Stray, F. (1998). The Natural Guide to Medicinal Herbs and Plants. Tiger Books International, London. Pp. 12-16.
[54]. Trease, G. E. and Evans, W. C. (1989). A Textbook of Pharmacognosy, 12th Edition. Bailliere Tindall, London. Pp. 45 - 50.
[55]. Uma, C. and Sekar, K. G. (2014). Phytochemical analysis of a folklore medicinal plant citrulluscolocynthis L (bitter apple). *Journal of Pharmacognosy and Phytochemistry*, **2** (6): 195 - 202
[56]. Veerapur, V. P., Prabhakar, K. R., Parihar, V. P., Kandadi, M. R. and Ramakrishana, S. (2009). *Ficusracemosa* Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector. *Evidence-Based ComplementaryandAlternative Medicine*,**6** (3): 317 - 324.
[57]. Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*,**64:** 555 – 559.

Obasi, D. C, etal. "Phytochemical, Nutritional and Anti-Nutritional Analyses of Ruzu Herbal Bitters". *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(1), (2020): pp. 04-17.