Original Research Article

Phytonutrients and Anti-nutrient Composition of aqueous extract of fermented seeds of *Prosopis Africana*

ABSTRACT

Prosopis africana is a perennial leguminous plant in the genus *Prosopis*. The seeds are used as food condiment. This study was aimed at evaluating the phytochemical, proximate, vitamin and anti-nutrient compositions of aqueous extract of fermented seeds of *Prosopis africana*. The analyses were done using standard biochemical methods. Assessment of the bioactive constituents was carried out using gas chromatography-mass spectrometry. The result of the phytochemical analysis revealed quantifiable levels of Steroid, phenols, triterpenes, alkaloids, flavonoids, glycosides, terpenoids and saponin. The result of the proximate analysis showed that fermented seeds of *Prosopis africana* contains moisture (50.51 ± 0.01%), ash (3.78± 0.004%), crude fat (4.45 ± 0.03%), crude fibre (2.49 ± 0.01%), crude protein (25.27 ± 0.07%) and Carbohydrates (13.56 ± 0.003%). The energy value was 195.37 Kcal/100g. Vitamins A (6.38 ± 0.001), vitamin C (36.77 ± 0.004), Vitamin D (0.43 ± 0.003) and vitamin E (0.84 ± 0.001) were present in the extract. The anti-nutrients detected were tannin (12.27 ± 0.06 mgGAE/kg), Phytate (3.68 ± 1.18 mg/kg), oxalate (44.36 ± 14.16 mg/kg), trypsin (4.72 ± 0.46 mg/kg) and cyanide (0.47 ± 0.14 ppm). A total of seventeen (17) bioactive constituents were found using gas chromatography-mass spectrophotometer which include 1,2-Benzisothiazol-3-amine, 2,5-Dihydroxyacetophenone, Cyclopentasiloxane, Propionic acid, Cyclohexasiloxane, 1,2-Bis(trimethylsilyl) benzene, Cycloheptasiloxane, 2-bromobutyloxychalcone, Cyclooctasiloxane, Fumaric acid, 5-Methyl-2-phenylindolizine, 4-(acridin-9—ylamino)-phenyl)-acetamide, 1,2-Bis(trimethylsilyl)benzene), 5-Methyl-2-phenylindolizine, 1,2-Benzisothiazole-3-propanoic acid, 1,4-Bis(trimethylsilyl) benzene and Cyclotrisiloxane. This study showed that aqueous extract of *Prosopis africana* seed contains various compounds which are found useful for nutritional and medicinal purposes.

Keywords: *Prosopis africana*, Phytochemical, Proximate, Vitamin, Anti-nutrient, Fermented.

1.0 Introduction

Prosopis africana is a leguminous tree and one of the forty four (44) species of the genus *Prosopis* [1]. It is one of the lesser known leguminous seed crops used as food condiment in Nigeria. It thrives well in the Savannah region of Nigeria although there is no record of yield of annual production [2]. The fermented seeds of *Prosopis africana* are ground into a paste, moulded in balls, and sun-dried before being consumed or sold [3]. In Nigeria, fermented seeds *Prosopis africana* are variously called *okpei* (*Igbo*), in some Igbo communities, it is called *ugba-nta*, *ayan* (*Yoruba*), *okpeye* (*Idoma and Tiv*) and *kiriya* or *kiriaya* (*Hausa*) [4]. The plant, which

is a quick-growing tree about 17m tall is native to intertropical Africa, occurring from Senegal to Ethiopia throughout the Sudanese and Guinean eco-zones, reaching the border of the Sahelian eco-zone to the north. *Prosopis africana* tree is valuable for its thick wood. The indehiscent pods are palatable to man and animals [5].

Traditional diets in West Africa often lack variety and consist of large quantities of staple food [6]. The staple foods provide the calories but are poor in other nutrients. Soups are the main sources of protein and minerals in food and diets. One of the ways to improve the diet has been to improve the nutrient content of soups. With high contents of protein, legume condiments can serve as a tasty complement to sauces and soups and can substitute for fish or meat [7]. Plant based foods offer an array of nutrients that are essential for human nutrition and promotion of good health. [8]. Processing techniques can increase the nutritional quality of the plant foods by reducing specific anti-nutrients [9].Traditional food systems are considered to be the backbone of modern food industries [10]. Food is an essential ingredient to life, and access to food is often the limiting factor in the size of a given population. The way to know which chemicals and how much of them are in food is through chemical analysis. Only then can the nutritional needs for the different chemicals or their effects on health be known. Most needs for food analysis arise from nutrition and health concerns, but other reasons for food analysis include process-control or quality-assurance purposes, flavour, palatability issues and checking for food adulterations [11]. Therefore, this study evaluated the nutritive and anti-nutritive composition of aqueous extract of fermented seeds of *Prosopis africana.*

2.0 Materials and Methods

2.1 Collection of plant material

Prosopis africana seeds were collected from its natural habitat, in Nsukka, Enugu State, Nigeria. It was identified by a botanist, Alfred Ozioko at Bio-resource and Development Conservative Programme (BDCP), Nsukka, Nigeria.

2.2 Sample Preparation

2.2.1 Fermentation of the plant seed sample:

Following the traditional method of fermentation, the seeds of *Prosopis africana* were boiled for up to six hours and allowed to cool to room temperature. The seed coats were removed by pressing between fingertips. These coats were later decanted along with the washing water leaving the clean seed cotyledons. The clean cotyledons were boiled for another 2hours. This allowed the seeds to become soft, reduced bitterness and made them easy for fermentation. The cotyledons were later drained through sieve and wrapped with paw-paw leaves. The wrapped cotyledons were put in clean bowls covered with the same leaves for a period of four days (for

fermentation to take place). After fermentation, the resultant product, which was brown in colour was *okpeye*, a strong-smelling mass of sticky cotyledons covered by a whitish mucilaginous film produced during fermentation [12]. The fermented seeds were ground in a mortar into a smooth paste. The *okpeye* was made into ball of 3-5 diameter, arranged in trays and dried for 1-2 days under the sun. The product became dark after sun drying.

2.2.2 Preparation of the aqueous extract of fermented *Prosopis africana* seeds.

The extraction was done by soaking the fermented *Prosopis africana* seed in water in the ratio of 1:5 for 24 hours at room temperature (26-28 $^{\circ}$C). The macerate was filtered using Whatman No 1. Filter paper and put in water bath at a temperature of 60 $^{\circ}$C to concentrate in order to obtain crude paste-like extract. It was thereafter stored in an air tight plastic container and kept in the refrigerator (4 °C) and used for the study.

2.3 Biochemical Assays

2.3.1 Phytochemical Analysis

Qualitative phytochemical analysis:

The qualitative phytochemical analysis of aqueous extract of fermented *Prosopis africana* seeds was carried out according to the methods of Harborne [13] and Trease and Evans [14] to identify its active constituents.

Test for alkaloids

A quantity, 0.2 g of the sample was boiled with 5 ml of 2% HCl on a steam bath. The mixture was filtered and 1ml aliquots of the filtrate were treated with 2 drops of the following reagents

(i) Dragendorff's reagent: An orange precipitate indicated the presence of alkaloids.
(ii) Mayer's reagent: A creamy-white precipitate indicated the presence of alkaloids.
(iii) Wagner's reagent: A reddish-brown precipitate indicated the presence of alkaloids.
(iv) Picric acid (1%): A yellow precipitate indicated the presence of alkaloids.

Test for flavonoids

A quantity, 0.2 g of the sample was heated with 10 ml ethyl acetate in boiling water bath for 3 minutes. The mixture was filtered, and the filtrate was used for the following tests.

(i) Ammonium test: 4 ml of the filtrate was shaken with 1ml of dilute ammonium solution to obtain two layers. The layers were allowed to separate. A yellow precipitate observed in the ammonium layer indicates the presence of flavonoids.

(ii) Aluminium chloride test: 4ml of the filtrate was shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow colouration that indicates the presence of flavonoids.

Test for saponins

A quantity (0.1g) of the sample was boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot. The filtrate was used for the following tests.

(i) Emulsion test: A quantity, 1 ml of the filtrate was added to two drops of olive oil. The mixture was shaken and observed for the formation of emulsion.

(ii) Frothing test: A quantity, 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

Test for glycosides

A quantity, (2.0 g) of the sample was mixed with 30 ml of distilled water and 15 ml of dilute sulphuric acid respectively and heated in a boiling water bath for 5 minutes. The mixtures was filtered and the filtrates used for the following test.

(i) To 5 ml of each of the filtrate 0.3 ml Fehling's solutions mixtures of A and B was added until it turned alkaline (tested with litmus paper) and heated on a boiling water bath for 2 minutes. A brick-red precipitate indicates the presence of glycosides.

Test for tannins

A quantity, 2 g of the sample was boiled with 5 ml of 45% ethanol for 5 minutes. The mixture was cooled and then filtered and the filtrate was treated with the following solutions.

(i) Lead sub acetate solution: To 1ml of the filtrate, 3 drops of lead sub acetate solution was added. A gelatinous precipitate indicates the presence of tannins.

(ii) Bromine water: To 1 ml of the filtrate was added 0.5 ml of bromine water and then observed for a pale brown precipitate.

(iii) Ferric chloride solution: a quantity, 1 ml of the filtrate was diluted with distilled water and then 2 drops of ferric chloride solution was added. A transient greenish to black colour indicates the presence of tannins.

Test for terpenoids and steroids

A known volume, 9 ml of ethanol was added to 1g of the sample and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1hour, and the waxy matter filtered off.

The filtrate was extracted with 2.5 ml of chloroform using a separating funnel. To 0.5 ml of the chloroform extract in a test tube was carefully added 1 ml of concentrated sulphuric acid to form a lower layer. A reddish-brown interface shows the presence of steroids. Another 0.5 ml aliquot of the chloroform extract was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on water. A grey colour indicates the presence of terpenoids.

Test for Glycoside

10 cm^3 of 50% H_2SO_4 was added to 1cm^3 of the extracts, the mixture was heated in boiling water for 15 minutes. 10cm3 of Fehling's solution was added and the mixture boiled. A brick red precipitate indicated the presence of glycosides.

Test for phenolics

2 drops of 5% FeCl, was added to 1Cm3 of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics.

Test for Triterpenes

5 drops of acetic anhydride was added 1cm3 of the extracts. A drop of concentrated H2SO4 was then added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue green colour indicates the presence of triterpenes.

Quantitative phytochemical analysis

Determination of tannins Content

The method of Swain [15] was used for the determination of the tannin content of *P. africana.* A quantity, 0.2 g of finely ground sample was measured into a 50 ml beaker. About 20ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80^oC for 1 hr and stirred with a glass rod to prevent bumping. The extract was filtered using a double layer of Whitman No. 1 filter paper into a 50 ml volumetric flask then 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10ml of 17% Na_2CO_3 were added and mixed properly. The mixture was made up to mark with distilled water and allowed to stand for 20 mins when a Mish-green colouration developed. Standard tannic acid solutions of range 0.10 mg were treated similarly as 1mI of sample above. The absorbances of the tannic acid standard solutions as well as samples were read after colour development at 760 nm. The tannin content was calculated using the formular:

Tannin (mg/100g) = <u>Absorbance of sample x Average gradient x Dilution factor</u>

Weight of sample x 1000

Determination of Coumarin

This was determined according to the method of Willard and Karl [16]. Add 0.5ml of 5N NaOH to the solution for 1ml of the extract (0.5g in 1ml of ethanol), heat at 80^{o}C for 5min, cool, add 0.75ml of 5N H_2SO_4, mix thoroughly, add 0.25g of anhydrous $NaHCO_3$, mix and transfer to the extractor. Rinse the flask with distilled water and transfer to the extractor and make up to 50ml extract for 3hrs with pet. Ether, remove the inner tube and transfer the pet ether in the extractor to the extraction flask. Add 20ml of water to the pet ether extract and carefully evaporate the pet ether in a water bath at 50-55^{o}C. Transfer the aqueous solution to a volumetric flask, make up to 50ml with continuous mixing. Pipette 25ml into a flask and add 1% Na_2CO_3 solution, heat in a water bath at 85^{o}C for 15min and cool. Add 5ml of the diazonium solution and let stand for 2 hours. Read the absorbance at 540nm against reagent blank. Calculate the coumarin content from the standard curve.

Determination of phenolic content

The total phenolic content of sample was estimated according to the Makkar et al., [17]. The aliquots of the extract was taken in a test tube and made up to the volume of 1ml with distilled water. Then 0.5ml of Folin-ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40min, and the absorbance was recorded at 725nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1 – 10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

Determination of Triterpenes

This was determined according to the method of Simonyan *et al* [18]. 0.50g of sample was weighed into a 50ml conical flask and 20ml of 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40ml of 10% sodium Dodecyl Sulphate (SDS) solution. 1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30minutes. Standard triterpenes of concentration range 0-5mg/ml were prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S>A. the absorbances of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wave length of 510nm.

The percentage of triterpenes was calculated using the formula:

$$\frac{\text{Absorbance of sample X Average gradient X Dilution factor}}{}$$

Weight of sample X 10,000

Determination of flavonoids

This was determined according to the method of Harborne [13]. A quantity, 5 g of the sample was boiled in 50 ml of 2 MHCl solution for 30min under reflux. It was allowed to cool and then filtered through Whatman No. 1 filter paper. A measured volume of the filtrate was treated with equal volume of ethyl acetate starting with drops. The solution was filtered into a weighed crucible and heated to dryness in an oven at 60^0C. The dried crucible was weighed again and the difference in the weight gave the quantity of flavonoid present in the sample.

Determination of alkaloids

The quantitative determination of alkaloid was described by Harborne [13]. A known quantity, 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 hours. This was filtered and the filtrate was concentrated down in a water bath to one – quarter (1/4) of the original volume. Concentrated ammonia was added drop-wise to the filtrate till a precipitate was formed. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue, the alkaloid, was dried and weighed.

Determination of Saponins

The method used was that of Obadoni and Ochuko [19]. The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 550C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^0C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n- butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as mg/g.

Determination of steroids

This was determined by the method described by Edeoga *et al*. [20]. A known weight of each sample was dispersed in 100 ml freshly distilled water and homogenized in a laboratory blender. The homogenate was filtered and the filtrate was eluted with 0.1 normal ammonium hydroxide solution (pH 9). The eluent (2 ml) was put in test tube and mixed with 2 ml of chloroform. Ice-

cold acetic anhydride (3 ml) was added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added. Standard sterol solution was prepared and treated as described above. The absorbances of standard and prepared sample were measured using spectrophotometer at 420 nm.

Determination of Terpenoid

This was done according to the method of Harborne [21]. A quantity 1g of the sample was macerated with 50mls of ethanol and then filtered. The filterate 2.5ml was added to 5% aqueous phosphomolybdic acid solution, conc. H_2SO_4 was added gradually. This was stood for 30mins and made up to 12.5ml with ethanol. The absorbance was read at 700nm.

Determination of glycosides

This was done according to the method of Harborne [21]. A quantity 1g of the sample was macerated with 20ml of distilled water, 2.5ml of 15% lead acetate was added and then filtered. A volume of 2.5ml of chloroform was added and the mixture shaken vigorously. The lower layer was collected and evaporated to dryness. The residue was dissolved with 3ml of glacial acetic acid. 0.1ml of 5% ferric chloride and 0.25ml of conc. H_2SO_4 were added, shaken and incubated for 2hrs in the dark. The absorbance was read at 530nm.

Determination of glycosides

This was done according to the method of Harborne [21]. A quantity 1g of the sample was macerated with 20mls of distilled water and filtered. To 1ml of the filtrate was added 1ml of alkaline copper reagent. This was boiled for 5mins and cooled. 1ml of phosphomolybdic acid and 2mls of distilled water were added and the absorbance at 420nm.

2.3.2 Proximate Analysis.

Determination of proximate composition of the fermented seeds of *prosopis africana* was analysed using the method of AOAC [22].

Determination of Protein:

The protein content was determined using micro kjeldahl method as described by Pearson [23]. A quantity of 0.5g of the sample was added 10mls of conc. Sulphuric acid and 1g of the catalyst mixture. Heated cautiously on digestion rack under fume hood until a greenish clear solution appears. Allowed to cool and make up to 50mls with distilled water. The digested sample was transferred into distillation apparatus and distilled. 10mls of the distillate was titrated with 0.1ml HCL to first pink colour.

$$\%\text{Protein} = \frac{\text{Titre} \times 14.01 \times 0.1\text{m} \times 100 \times 6.25 \times 50}{}$$

100 X 0.5g X 10

Determination of Moisture Content (Oven Method):

2g of the sample was weighed inside a clean dried crucible, the constant weight dried at 60^{o}C in a hot stimulating ovum for 24hrs, cooled inside a desiccator and weighed, then the crucible was washed, dried in the ovum and empty weight of the crucible taken.

$$\%\text{Moisture} = \frac{\text{Difference in weight}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of Ash Content:

The crucible was heated at 600^{o}C, cool and weighed, 2g of the sample was transferred into a weighed crucible, and the content placed into a muffle furnace and ashed at 600^{o}C for 3hrs. It was allowed to cool inside a desiccator and weighed.

$$\%\text{Ash} = \frac{\text{Difference in weight}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of Crude Fibre:

2g of the sample was defatted with petroleum ether, boil under reflux for 30minutes with 200mls of a solution containing 1.25g of H_2SO_4 per 100mls of solution. Filtered with cheese cloth, washed with boiled water until the washing are no longer acidic. The residue was transferred to a beaker and boiled for 30minutes with 200mls of a solution containing 1.25g of carbonate free sodium hydroxide per 100mls. Then filtered and transferred into a crucible. The residue was dried in the ovum and weighed. Then the sample was ashed at 600^{o}C in a muffle furnace and the dried weight taken.

$$\%\text{Crude Fibre} = \frac{\text{loss in weight}}{\text{After incineration}} \times \frac{100}{1}$$

Determination of Fats (Soxhlet Method):

250 mls of clean boiling flask was dried in the ovum at 105^{o}C for 30minutes. 2g of the sample was transferred into the flask. 300mls of petroleum ether was added. The Gimble was plugged and the extraction thimble covered with cotton wool and the soxhlet apparatus assembled and refluxed for 6hours. The thimble was removed and the petroleum ether in the top container of the set up and drain for another extraction. The petroleum ether layer collected into a beaker and dried in the ovum and weighed. The beaker was washed and also dried in the ovum to get the empty weight.

$$\%Fat = \frac{\text{Difference in weight}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Determination of Carbohydrate.

The carbohydrate content was determined using difference method. Thus;

Carbohydrate = 100 (%Fats + %Ash + Moisture + 2 Protein +%Crude Fibre)

2.3.3 Anti-nutrient content Analysis

Determination of Oxalate

Oxalate was determined by the method reported by Munro [24]. One gram of the sample was placed in a 250 ml volumetric flask suspended in 190 ml of distilled water. A known volume, 10 ml of 6M HCl solution was added to the sample and the suspension digested at 100 C for 1h. The sample was then cooled and made up to 250 ml mark of the flask. This was filtered after which a duplicate portion of 125 ml of the filtrate was measured into a beaker, and four drops of methyl red indicator were added, followed by the addition of concentrated NH_4OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90^{o}C, cooled, and filtered to remove the precipitate containing ferrous ion. Each of the filtrates was again heated to 90^{o}C and 10 ml of 5% $CaCl_2$ solution was added to each of the samples whilst stirring consistently. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatants were decanted and the precipitates completely dissolved in 10 ml 20% H_2SO_4. The total filtrate resulting from the digestion of 1g of the sample was made up of 200 ml. Aliquots of 125 ml of the filtrate were heated until near boiling and then titrated against 0.05M standardized KMnO4 solution to a pink colour which persisted for 30s. The oxalate content of the sample was calculated.

Determination of Phytate

Phytate was determined through phytic acid determination as described by Lucas and Markaka [25]. This entails weighing 1 g of the sample into a 250 ml conical flask. Then, 100 ml of 2% concentrated HCl was used to soak the sample in the conical flask for 3 h and then filtered through a double layer filter paper. A known volume, 50 ml of the sample filtrate was placed in a

250 ml beaker and 107 ml of distilled water added to ensure proper acidity. Moments later, 10 ml of 0.3% ammonium thiocyanate solution was added as an indicator to each sample solution and titrated with standard iron chloride solution which contained 0.00195 g iron/ml and the endpoint was signified by a brownish-yellow colouration that persisted for 5 min. The percentage of phytic acid was then calculated.

Determination of Hydrogen cyanide

The method of Onwuka [26] was adopted in Cyanogenic glycoside determination. One gram of the sample was weighed, added to 50 ml distilled water in a conical flask, and allowed to stand overnight. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated in a water bath for 5 min. The absorbance of the samples was taken at 490 nm, with that of a blank containing 1 ml distilled water and 4 ml alkaline picrate solution before the preparation of the cyanide standard curve. A colour change from yellow to reddish-brown after incubation for 5 min indicates the presence of hydrogen cyanide and was calculated from the standard curve.

Determination of Trypsin inhibitor

Trypsin inhibitor was determined by the method reported by Prokopet and Unlenbruck [27]. One gram of the sample was dispersed into 50 ml of 0.5M NaCl solution. The mixture was stirred for 30 min at room temperature and centrifuged at 1500 rpm for 5 min. The supernatant was filtered, and the filtrate was used for the assay. To the substrate of the sample, 2 ml of the standard trypsin solution was added. The absorbance of the mixture was taken at 410 nm using 10 ml of the same substrate as blank.

2.3.4 Vitamin Analysis

Vitamins were determined using the methods outlined by AOAC [22].

Vitamin A concentration

A quantity, 1 g of the samples was weighed accurately into 100 ml flask fitted with reflux condenser. Then 10 ml absolute alcohol and 20 ml alcoholic sulphuric acid were added. The condenser and flask were wrapped with aluminum foil. They were then refluxed for 45 min and cooled. Subsequently, 5 ml of water was poured into each flask and relocated to a separator funnel. Non-saponified matter was extracted with 30 ml of diethyl ether. The combined ether extract was then washed free from acid and dried over anhydrous sodium sulphate. The extract was evaporated at low temperature while protecting them from sunlight, final traces of solvent being removed in a stream of nitrogen and then residues dissolved immediately in 10 ml

isopropanol. The extinction of the freshly prepared extract in isopropanol was read at 325 nm against a solvent blank (T1). The cuvettes were then removed, exposed to UV light until the extinction no longer fell with time and then the absorbance recorded (T2). The standard vitamin A solution was treated the same way (ST1 – ST2).

$$\text{Vitamin A (mg/100mg)} = \frac{T1 - T2}{ST1 - ST2} \times 1 \times \text{Dilution factor}$$

Vitamin E concentration

One gram of the ground sample was measured into 100 ml flask and 10 ml of absolute alcohol (ethanol) was added. Twenty millilitres of 1 M alcoholic sulphuric acid and 18 ml of concentrated H_2SO_4 in 1 L of ethanol were added and refluxed for 45 min and cooled in a reflux condenser. A volume of 10 ml of the clear solution was pipette into a test tube and heated in a water bath at 90^oC for 30 min and allowed to cool standard and a blank were prepared and the absorbance read at 470 nm. Vitamin E was calculated.

Vitamin E (mg/100g) = Absorbance x Dilution factor

Vitamin C concentration

Ascorbic acid was determined by titration with diphenol indo 2, 6 – dichlorophenol (DPIP). The powdered sample (0.2 g) was mixed with 4 ml of a buffer solution made up of 1 g/l oxalic acid and 4 g/l sodium acetate anhydrous. This was titrated against a solution containing 295 mg/l DPIP and 100 mg/l sodium bicarbonate. Vitamin C content of the samples was calculated.

$$\text{Vitamin C (mg/100g)} = \frac{MV \times 100 \times 100}{10B}$$

M = mass of ascorbic acid titrimetric equivalent to 0.001 M DPIP solution (mg)

100 is the dilution ratio of the sample taken, the second 100 is the scaling factor for conversion to per 100 g of raw material, 10 is the titrate volume.

V = titrant volume (0.00 1 M DPIP solution) ml

B = weight of the sample extract used

Vitamin D concentration

One gram of the sample was weighed into a flat bottom flask. This was followed by the addition of 1g pyropanol and 25 ml ethanolic potassium hydroxide solution (60 ml ethanol, 30 ml 50% potassium hydroxide). This was extracted thrice with petroleum ether followed by washing with water. The sample was then filtered and then evaporated to dryness in the water bath.

Afterwards, 1 ml 11N HCL and 1 ml trichloromethane were added to the dried extract. The volume of the mixture was made up to 7 ml with acetone and then read at 450 nm in the spectrophotometer. The absorbance obtained from the sample extract was converted to cholecalciferol concentration by means of a calibration curve generated using different concentrations of vitamin D.

2.3.5 Assessment of Bioactive constituents of aqueous extract of fermented *Prosopis africana* seeds using gas chromatography-mass spectrometry.

The GC analysis was carried out in AGILENT 6890 gas chromatography with a fused GC column (OV-101) coated with polymethyl silicon (0.25nm X 50m) and the conditions were as follows: temperature programming from 80 – 200 ^{o}C held at 80 °C for 1 minute, rate 5^{o}C/min and at 200 °C for 20min. Flame ionization detector (FID) temperature at 300 °C, injection temperature of 220 °C and carrier gas nitrogen at a flow of 1ml/ min, split ratio 1:75, GC-MS analysis was conducted using AGILENT 6890 gas chromatography with injector temperature of 230°C and carrier gas pressure of 100kpa. The column length was 30m with a diameter of 0.25mm and the flow rate of 50ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2sec. The mass spectrum was also equipped with a computer fed mass nspectra data bank. Identification of compounds was performed according to their mass spectra (NIST v 1.7) National Institute of Standards and Technology. Positive identification was assumed when good matches (90% and more) of mass spectra were achieved.

3.0 RESULTS

3.1 Proximate Composition

Table 1 shows the proximate composition of the aqueous extract of fermented seeds of *Prosopis africana* (*Okpeye*). The seeds contains very high amount of moisture (50.51 ± 0.01) and very small amount of crude fibre (2.49 ± 0.01). It also contained appreciable amount of energy.

Table 1: Proximate Composition of Aqueous Extract of Fermented Seeds of *Prosopis africana*.

Parameters	Composition %
Moisture Content	50.51 ± 0.01
Ash Content	3.78 ± 0.004

Crude Fat	4.45 ± 0.03
Crude Fibre	2.49 ± 0.01
Crude Protein	25.27 ± 0.07
Carbohydrate	13.56 ± 0.003
Energy (Kcal/100g)	195.37 (Kcal/100g)

Values are mean ± standard deviation

3.2 Phytochemical Composition

Table 2 shows the data obtained from the qualitative and quantitative phytochemical analysis of aqueous extract of fermented seeds of *Prosopis africana* (*Okpeye*). The result indicated that Saponin, Tanin, Phenolics, Steroids, Glycosides, Flavonoids, terpenoids, triterpenes and alkoloids were present. Steroids was the highest constituent found while terpenoids was the lowest constituent found.

Table 2: Qualitative and quantitative phytochemical Composition of Aqueous Extract of Fermented Seeds of *Prosopis africana* (*Okpeye*).

Phytochemicals	Qualitative Composition	Concentration (mg/g)	Concentration (%) value
Saponin	+	0.35 ± 0.00	0.08
Tanin	+	12.02 ± 0.44	2.87
Phenolics	+	98.85 ± 0.41	23.55
Steroids	+	166.00± 0.47	39.54
Coumarin	-	ND	ND
Glycoside	+	8.59± 0.01	2.05
Flavonoids	+	17.25± 0.04	4.11
Terpenoids	+	0.73± 0.05	0.17

Triterpene	+	79.00 ± 0.47	18.82
Anthocyanin	-	ND	ND
Phlobatanin	-	ND	ND
Alkaloids	+	36.97 ± 0.06	14.39

Values are mean ± Standard Deviation. Key: + (Present); - (Not Present); ND(Not Detected)

3.3 Vitamin Composition

Table 3 shows the result of the vitamin analysis. The result shows that the sample is very rich in vitamin C and A. Others were present in very low amount.

Table 3: Vitamin Composition of Aqueous Extract of Fermented Seeds of *Prosopis africana* (*Okpeye*).

Vitamins	Concentration (mg/g)	Concentration (%) of total value
Vitamin A	6.38 ± 0.001	14.4
Vitamin C	36.77 ± 0.004	82.8
Vitamin D	0.43 ± 0.003	0.9
Vitamin E	0.84 ± 0.001	1.9

Values are mean ± Standard Deviation.

3.4 Anti-nutrient Composition

Table 4 shows the result of the anti-nutrient analysis of aqueous extract of fermented seeds of *Prosopis africana* (*Okpeye*). The result shows that shows that aqueous extract of fermented seeds of *Prosopis africana* (*Okpeye*) contains traces of anti-nutrients. The oxalate was the highest constituent while cyanide was the lowest constituent.

Table 4: Anti-nutrient composition of Aqueous Extract of Fermented Seeds of *Prosopis africana* (*Okpeye*)

Anti-nutrient	Concentration
Tannin (mgGAE/kg)	12.27± 0.06
Phytate (mg/kg)	3.68 ± 1.18
Oxalate (mg/kg)	44.36 ± 14.16
Trypsin(mg/kg)	4.72 ± 0.46
Cyanide (ppm)	0.47 ± 0.14

3.5 Bioactive Constituents

Table 5 Shows the identified compounds in aqueous extract of fermented *Prosopis africana* seeds (*Okpeye*) using gas chromatography-mass spectrophotometry (GC-MS) The GC-MS (gas chromatography-mass spectrophotometry) of aqueous extract of fermented seeds of *Prosopis africana* recorded a total of seventeen (17) peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, Peak area(%), height and mass spectral fragmentation patterns to that of the known compounds described by National Institute of Standards and Technology (NIST) Library.

Table 5: Bioactive constituents identified in the aqueous extract of fermented *Prosopis africana* seeds using gas chromatography-mass spectrometry.

Peak #	Retention Time (min)	Compound Name	Area
1	3.834	1,2-Benzisothiazol-3-amine	1.22
2	5.863	2,5-Dihydroxyacetophenone	1.57
3	8.511	Cyclopentasiloxane	15.04
4	9.299	Propionic acid	3.64
5	10.285	Cyclohexasiloxane	20.79
6	11.271	1,2-Bis(trimethylsilyl) benzene	0.95

7	11.581	Cycloheptasiloxane	17.93
8	11.976	2-bromobutyloxychalcone	2.05
9	12.680	Cyclooctasiloxane	8.98
10	13.412	Fumaric acid	6.86
11	13.609	5-Methyl-2-phenylindolizine	4.27
12	13.947	4-(acridin-9—ylamino)-phenyl)-acetamide	5.05
13	14.145	1,2-Bis(trimethylsilyl)benzene)	1.04
14	14.398	5-Methyl-2-phenylindolizine	2.11
15	14.680	1,2-Benzisothiazole-3-propanoic acid	4.31
16	15.300	1,4-Bis(trimethylsilyl) benzene	3.08
17	16.398	Cyclotrisiloxane	1.12

4.0 Discussion

Fermented condiments remain the key constituents of diets throughout many parts of Africa. Soups are the main source of protein and minerals and one of the ways to improve the diet is to improve the nutrients in the soup through the use of condiments such as the fermented legume seeds [28]. The result of the proximate analysis of the fermented seeds of *Prosopis africana* in table 1 shows that it is high in moisture (50.51%). Moisture content is the amount of water in a material. It is an index of shelf life of a food stuff. The moisture content of fermented seeds of *Prosopis africana* shows that it has a relatively low shelf life and may not be stored for a long time without being susceptible to microbial attack. Also, the high moisture content could be as a result of the *Prosopis africana* seeds being fermented to *okpeye*, which enhances metabolic activities such as those of fermentation. Fermentation markedly improves shelf life, digestibility, nutritive value and flavours of the raw seeds. When the seeds are fermented, they become tasty and protein-rich. They are added to dishes directly or used as thickening agents in soups and stew [28]. In addition to water, a normal diet must provide metabolic fuels (mainly carbohydrates and lipids), protein (for growth and turnover of tissue protein, as well as a source of metabolic fuel and fibre (for bulk in the intestinal lumen). Globally under-nutrition is widespread. It leads to impaired growth, defective immune system, and reduced work capacity. From the result of the

proximate analysis, the fermented seeds of *Prosopis africana (Okpeye)* contains carbohydrates (13.5%) with appreciable amount of energy (195.37kcal/ 100g). With this amount of carbohydrate, it can be considered as a potential source of energy. The ash content was 3.78%. Ash is an index of mineral content of a material. The ash content of fermented seeds of *Prosopis africana* is relatively high and this value shows the percentage of inorganic mineral elements present. It is known that high mineral elements in foods enhances growth and development and also catalyses metabolic processes in human body [29]. The crude fat content was 4.45%. This value suggests that the seed of *Prosopis africana* cannot be referred as oil seed. Fats are esters of fatty acid with glycerol. Fats could provide the body with enough amount of energy and aid in such processes as absorption of vitamins. Dietary supplementation of long chain ω3 fatty acids is believed to have beneficial effects in a number of chronic diseases, including cardiovascular disease, rheumatoid arthritis and dementia [30]. The crude protein content obtained was 25.27%. This is relatively high. Dietary protein is required by humans because it contributes to the body's supply of indispensable amino acids and to its supply of nitrogen for the synthesis of the dispensable amino acids. Plant protein foods contribute about 65% of the per capita supply of protein worldwide [31]. A high intake of protein does not lead to positive nitrogen balance; although it increases the rate of protein synthesis. It also increases the rate of protein catabolism so that nitrogen equilibrium is maintained. Because growing children need proteins in the body for them to grow, they have a proportionally greater requirement than adults and should be in positive nitrogen balance [30]. The crude fibre content was found to be 2.49% which was relatively low. Dietary fibre refers to non- digestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants [32]. Adequate intake of fibre, has been implicated in the treatment of several gastrointestinal disorders, including diverticular disease, gall stones, irritable bowel syndrome and constipation [31].

The result of the quanlitative and quantitative phytochemical analyses in Table 2 shows the presence of saponins, tannins, phenolics, steroids, glycosides, flavonoids, terpenoids, triterpenes and alkaloids. Steroid (166.00mg/g) was the most abundant followed by phenolics (98.85mg/g), triterpenes (79.00mg/g), alkaloids (36.97mg/g), flavonoids (17.25mg/g), tannins (12.04mg/g), glycosides (8.59mg/g), terpenoids (0.73mg/g) and the least which was saponins (0.35mg/g). Sterols are monohydroxy alcohols of steroidal structure. In the body, this sterol is an essential component of cell membranes, particularly the membranes of nerve tissue. Although cholesterol is probably best known for its association with atherosclerosis and heart disease, it has a number of essential roles in the body. It serves as the precursor for many other important steroids in the body, including bile acids, adrenocortical hormones, sex hormones, vitamin D and cardiac glycosides [30]. Phenols play a vital role in scavenging of free radicals. For many years, polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage through scavenging of free radicals. [33]. They possess antioxidant properties and may possibly have anticarcinogenic properties [34]. Steroid containing compounds are important in pharmacognosy due to their relationship with sex hormones [35]. Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes

mellitus [33]. Significant progress has been made in the field of cardiovascular diseases, and today it is well established that some polyphenols, administered as supplements or with food, do improve health status, as indicated by several biomarkers closely associated with cardiovascular risk. A considerable body of literature supports a role for oxidative stress in the pathogenesis of age-related human diseases and a contribution of dietary polyphenols to their prevention [33]. Plants produce primary and secondary metabolites which encompass a wide array of functions. Primary metabolites which include amino acids, simple sugars, nucleic acids and lipids are compounds that are necessary for cellular processes. Secondary metabolites include compounds produced in response to stress, such as the case when acting as a deterrent against herbivores [36]. Among all the compounds derived from biomass, terpenes have emerged as viable compound to serve as building blocks for the synthesis of polymers. Terpenes are merely hydrocarbons. They are produced mainly by plants, where they play a vital role in basic intra and intercellular processes, such as photosynthetic light reactions, or respiratory chains [37]. Pharmaceutical and food industries have exploited terpenes for their potentials and effectiveness as medicines and flavour enhancers. *Okpeye* is found to last for a longer period even after fermentation, this could be as a result of terpene having antimicrobial activities, terpenes is important due to the increase in antibiotic resistant bacteria [36]. Triterpenes are composed of three or six isoprene units and some studies have shown that there is promising potential for the use of triterpenes for people with diabetes by aiming to reduce glucose levels. Numerous *in vitro* and *in vivo* studies have revealed the multidirectional properties of triterpenes; anti-cancer, antioxidant, anti-inflammatroy, anti-atherosclerotic or antiviral [38]. Alkaloids are nitrogen-containing compounds that occur naturally and are reported to possess antimalarial, anticancer, antiasthma, antiarrhythmic, vasodilatory, analgelsic, hypoglycemic, antibacterial activities [39]. Alkaloids have pharmacological applications as anesthetics and CNS (Central Nervous System) stimulants [40]. Flavonoids are important group of polyohenols widely distributed among the plant flora [41]. Consumption of foods containing flavonoids has been linked to numerous health benefits once consumed and absorbed. Flavonoids act favourably in the body through actions such as inhibiting xanthine oxidase and arachidonic acid metabolism[42]. Flavonoids have an anti-inflammatory capacity since they inhibit the production of inflammatory mediators by modulating the arachidonic acid pathway, inhibiting several enzymes such as ATpase, prostaglandin, cyclooxygenase, lipoxygenase, NADH Oxidase, protein kinase, hydrolases, peroxidases, metallopeptidases, tyrosinases, and phopholipases [43]. Flavonoids also possess antioxidant properties and are reportedly involved in several physiological activities such as anti-inflammatory, anti allergic, anti-oxidant, anti-microbial, anti-diarrhoeal and anti-cancer [39]. Tannins are natural products found in most higher plant. Tannins are also considered as one of the effective components contributing to the fact that the risk of suffering from cardiovascular diseases and some forms of cancer can be reduced [44]. In medicine, tannin-containing plant extracts are used as astringents, against diarrhoea, as diurectics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, haemostatic pharmaceuticals [45]. Tannin have been implicated in the speeding up blood clotting processes, reduction of blood pressure, modulation of immune response and in reduction of plasma lipid [39]. Glycosides are non-

reducing organic componds. Glycosides are used in the treatment of heart disease e.g congestive heart failure and arrhythmia. Increasing the force of contraction of the heart is very important for most heart failure patients [46]. Saponins are natural surfactants or detergents, found in many plants. In the diet, phytochemical saponins have a wide spectrum of activity as anti-fungal and anti-bacterial agents, lowering of blood cholesterol, and inhibition of cancer cell growth saponins act by binding with bile acids and cholesterols so it is thought that these chemicals clean or purge these fatty compounds from the body, lowering the blood cholesterol levels. Some saponins affect the heart and have been used for over 100years to treat heart conditions [47]. From the result of the phytochemical screening, the presence of these phytochemicals in the fermented seeds of *Prosopis africana (Okpeye)* shows that they are of therapeutic and health importance.

From the result of vitamin analysis, the fermented seeds of *Prosopis africana (Okpeye)* is rich in vitamins all of which have important role to play. Vitamins are essential organic compounds required in very small amounts (micronutrients) that are involved in fundamental functions of the body such as growth, maintenance of health and metabolism [31]. The evidence presented in Table 3 revealed that vitamin C was the most abundant with a concentration of (36.77mg/g) which is 82.8% of the total vitamin content of the seed. Vitamin C is a significant antioxidants that protects the cell membranes from oxidative stress/ damage caused by free radicals [48]. Vitamin C has a different mechanism of action which depends on the conditions and hence can act as a pro-oxidant, a metal chelator, an oxygen scavenger or a reducing agent. Vitamin C is required for wound healing maintenance of normal connective tissues, promotes the absorption of dietary iron from the intestine and prevents development of scurvy [49]. Vitamin A was the second highest vitamin content with a concentration of (6.38mg/g). Vitamin A helps to provide good vision, healthy immune system and cell growth. Vitamin A possesses anti-cancer property through inhibition of DNA synthesis in cancer cells. It also delays tumour growth and inhibits division of leukaemia cells [50]. In the same vein, beta carotene, a precursor of vitamin A is very important in strengthening the immune system. Diets that are rich in beta carotene are said to aid in slowing down ageing and may also repair and protect DNA [51]. Vitamin E and D were the third and the fourth with concentrations of 0.8mg/g and 0.43mg/g respectively. Vitamin E known as anti-sterility vitamin is crucial in the development and normal functioning of the red blood cell and muscles [52]. Vitamin D is responsible for increased intestinal uptake of phosphate, magnesium and calcium as well as many other biological effect [53]. Antinutrients may exert beneficial health effect at low concentrations. Although people's sensitivity to anti-nutrients widely differs, adequate food processing is initially recommended to reduce anti-nutritional factors [54]. Table 4 revealed that there were anti-nutrients in the aqueous extract of fermented seeds of *Prosopis africana (Okpeye)*. Tannins (12.27 mgGAE/kg), phytate (3.68 mg/kg), oxalate (44.36 mg/kg), trypsin (4.72 mg/kg) and cyanide (0.47 ppm) were detected. Removing undesirable food components is essential for their quality improvement. Different techniques such as soaking, cooking, fermentation help in anti-nutritional disabling. Combination of several of the above mentioned methods may be more effective in removing anti-nutrients than using a sole technique [55]. Although minerals may be present in foods, they are not bioavailable unless the body absorbs them. Anti-nutritional components such as phytates and oxalates limit their

absorption [56]. The minerals in food have resulted in making use of a processing technique known as extrusion cooking [57, 58]. Extrusion cooking improves the nutritive value by reducing the levels of heat labile, non-nutritive compounds [59].

The GC-MS (gas chromatography-mass spectrophotometry) of aqueous extract of fermented seeds of *Prosopis africana* recorded a total of seventeen (17) peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, Peak area(%), height and mass spectral fragmentation patterns to that of the known compounds described by National Institute of Standards and Technology (NIST) Library. The phyto-constituents in the aqueous extract of fermented seeds of Prosopis africana were found to be 1,2-Benzisothiazol-3-amine, 2,5-Dihydroxyacetophenone, Cyclopentasiloxane, Propionic acid, Cyclohexasiloxane, 1,2-Bis(trimethylsilyl) benzene, Cycloheptasiloxane, 2-bromobutyloxychalcone, Cyclooctasiloxane, Fumaric acid, 5-Methyl-2-phenylindolizine, 4-(acridin-9—ylamino)-phenyl)-acetamide, 1,2-Bis(trimethylsilyl)benzene), 5-Methyl-2-phenylindolizine, 1,2-Benzisothiazole-3-propanoic acid, 1,4-Bis(trimethylsilyl) benzene, Cyclotrisiloxane. These identified compounds have been reported to possess different pharmaceutical and therapeutic activities such as anti-inflammatory, anti-oxidant, anti-microbial and anti-cancer activities, making them of special pharmaceutical importance. Fumaric acid is used as a nutritional additive and acidulant in various forms in the food and farming industries. It is the strongest lasting food acidulant that can control the growth of microorganisms, adjust pH and enhance flavour. The hydrophobic nature of fumaric acid results in persistent, long lasting sourness and flavour impact [60]. Fumaric acid is a naturally occurring organic acid. Many microorganisms produce fumaric acid in small amounts, as it is a key intermediate in citrate cycle. In addition to polymerization, there are two potentially new applications for fumaric acid. The first is as a medicine to treat psoriasis, a skin condition. Psoriatic individuals are unable to produce fumaric acid in their body due to a certain biochemical defect that interferes with adequate fumaric acid production in the skin. Therefore, Psoriatic individuals need to take orally fumaric acid in the form of fumaric acid monoethyl or dimethyl ester to treat their disease. In the field of fumaric acid production by fermentation, there are many aspects determining the productivity of the fermentation process, such as the applied microbial strain and its morphology, the use of a neutralizing agent [61]. Siloxanes are adaptable species that have found extensive application as versatile materials for functionalizing various surfaces and as a building blocks for polymers and hybrid organic-inorganic systems. Regarding the applications of the various polysiloxane materials, medical applications, such as for the manufacture of low-fouling and bacteria-resistant surfaces, are the most prominent [62]. Propionic acid is a chemical intermediate which is mostly used as an antimicrobial agent, anti-inflammatory factor, it is also has an analgesic and antipyretic properties. It enhances artificial flavours and fragrances. Propionic acid is beneficial to the human body and may play a role in satiety and energy homeostasis by specific mechanisms including activation of free fatty acid receptors, reducing lipogenesis level and glucose homeostasis. Propionic acid can serve as food additive applied to produce characteristic holes and nutty flavour. Propionic acid is a relatively strong organic acid, which can serve as an anti-

microbial agent in foodstuffs [63]. Acetamide derivative have been found to possess analgesic activity. Anagelsics, are most widely used drugs for pain [64].

Conclusion

In conclusion, the findings from this study have shown that fermented seeds of *Prosopis africana* contained rich amount of nutrients and phytochemicals and could be a potential source of therapeutic compounds. Generally, anti-nutrients reduce the assimilation of the essential nutrients by the body required for metabolic processes. However, the minute amount of the selected anti-nutrients analysed in fermented seeds of *Prosopis africana* was generally low and would not cause any health concerns.

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