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Phytonutrients and Anti-Nutrient Composition of Aqueous Extract of Fermented Seeds of *Prosopis africana*

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Authors' contributions

This work was carried out in collaboration among all authors. Author EIA conceived the work. Author OFCN wrote the protocol and designed the study. Authors EIA and ONA managed the analyses of the study, the literature searches, wrote the first draft of the manuscript and performed the statistical analysis. All authors read and approved the final manuscript.

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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

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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. INTRODUCTION

Prosopis africana is a leguminous tree and one of the forty four (44) species of the genus *Prosopis* [1]. It is among the leguminous plants which are less known and are used in Nigeria as food condiment. In Nigeria, it grows well in the Savannah region although, no annual yield production is recorded [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 originates from tropical Africa such as Senegal, Ethiopia, Sudanese, Guinea as well as Sahelian ecozone border to the north. This plant grows fast at about 17m tall and has a thick wood which makes it most valuable. The indehiscent pods are palatable to man and animals [5].

Most times, West African traditional diets lack variety and contains more of staple foods [6], which are less in other nutrients but are rich in calories. Proteins and minerals are the major nutrients found in soups. Increasing the nutrient value of soups is one of the many ways to enhance diet. Legume condiments are very tasty with high protein content and can replace fish or meat in soups and sauces [7]. Foods from plant offer a wide range of nutrients that are required in promoting good health and adequate nutrition [8]. Proper processing methods of plant foods can enhance the nutritional quality through reduction of certain anti-nutrients [9]. Traditional food systems are considered to be the backbone of modern food industries [10]. Food is an important component of life and accessibility to food is limited by population size. Food analysis is carried out in order to identify the chemicals contained in food. This will help to know the dietary needs of different chemicals that were identified and its possible effects on human health. Health and nutrition concerns as well as quality-assurance or process-control are the reasons for analysis of food. Others include flavour, palatability issues and examining for food adulterations [11]. Therefore, this study evaluated the nutritive and anti-nutritive composition of aqueous extract of fermented seeds of *Prosopis africana.*

2. 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 (25 ºC). 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 inches, 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 ˚C). The macerate was filtered using Whatman No 1. Filter paper and put in water bath at a temperature of 60 ˚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

2.3.1.1 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.

2.3.1.1.1 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.

2.3.1.1.2 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.

2.3.1.1.3 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.

2.3.1.1.4 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.

2.3.1.1.5 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.

2.3.1.2 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.

2.3.1.2.1 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.

2.3.1.2.2 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.

2.3.2 Quantitative phytochemical analysis

2.3.2.1 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°C 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 1ml 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) = Absorbance of sample x Average gradient x Dilution factor / Weight of sample x 1000

2.3.2.2 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°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°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°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.

2.3.2.3 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.

2.3.2.4 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:

Absorbance of sample X Average gradient X Dilution factor / Weight of sample X 10,000

2.3.2.5 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.

2.3.2.6 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.

2.3.2.7 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.

2.3.2.8 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.

2.3.2.9 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.

2.3.2.10 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.

2.3.2.11 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.

2.3.3 Proximate analysis

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

2.3.3.1 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.

%Protein = Titre X 14.01 X 0.1m X 100 X 6.25 X 50 / 100 X 0.5g X 10

2.3.3.2 Determination of moisture content (oven method)

2g of the sample was weighed inside a clean dried crucible, the constant weight dried at 60°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.

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

2.3.3.3 Determination of ash content

The crucible was heated at 600°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°C for 3hrs. It was allowed to cool inside a desiccator and weighed.

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

2.3.3.4 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°C in a muffle furnace and the dried weight taken.

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

2.3.3.5 Determination of fats (Soxhlet method)

250 mls of clean boiling flask was dried in the ovum at 105°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.

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

2.3.3.6 Determination of carbohydrate

The carbohydrate content was determined using difference method. Thus;

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

2.3.4 Anti-nutrient content analysis

2.3.4.1 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°C, cooled, and filtered to remove the precipitate containing ferrous ion. Each of the filtrates was again heated to 90°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" [65].

2.3.4.2 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" [65].

2.3.4.3 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".

2.3.4.4 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" [65].

2.3.5 Vitamin analysis

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

2.3.5.1 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)" [65].

Vitamin A (mg/100mg) = (T1 – T2 / ST1 – ST2) X 1 X Dilution factor

2.3.5.2 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°C for 30 min and allowed to cool standard and a blank were prepared and the absorbance read at 470 nm. Vitamin E was calculated" [65].

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

2.3.5.3 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" [65].

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

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

2.3.5.4 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" [65].

2.3.6 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°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. 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.

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.

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.

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 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)

4. DISCUSSION

In many places in Africa, food condiments that are fermented are the major components of diets. Proteins and minerals are the major nutrients found in soups. Increasing the nutrient value of soups by adding fermented legume seeds as condiments is one of the many ways to enhance diet. [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. The digestibility, flavours, nutrient value and shelf life of the raw seeds can be remarkably improved by fermentation. The taste of fermented seeds is enhanced while the protein contents are increased. These fermented seeds are used as thickeners in stew and soups or can be added directly [28]. Other than water, a normal diet must supply energy (majorly lipids and carbohydrates), protein (tissue protein turnover, growth and energy) and fibre (for easy peristalsis in the intestinal lumen). Globally under-nutrition is widespread. It leads to growth impairment, immune system defects and decreased work capability. 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 recommended as a potential energy source. The ash content was 3.78%. Ash indicates the amount of mineral in 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 improves development and growth as well as aids in catalysis of metabolism in the 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 can supply tangible quantity of energy and help in other body processes such as absorption of vitamins. Long chain omega-3 supplementation of diets is useful in so many chronic diseases such as rheumatoid arthritis, dementia and cardiovascular diseases [30]. The crude protein content obtained was 25.27%. This is relatively high. Humans need dietary supplementation of protein because it helps to supply the body with essential amino acids and nitrogen for the synthesis of non-essential amino acids. Protein foods of plant origin contribute about 65% of the per capita supply of protein worldwide [31]. An increase in protein intake does not lead to positive nitrogen balance., but it can increase the rate of protein synthesis and degradation in order to maintain nitrogen equilibrium. Because growing children need proteins in the body for them to grow, they have higher needs proportionally 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 is the indigestible lignin and carbohydrate that are not broken down and native 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].

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

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

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

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

(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. This sterol is an important constituent of the nerve tissues (cell membrane) in the body. There is an association between cholesterol and cardiovascular diseases but it has a lot of vital functions 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 are known free radical scavengers. Antioxidants and polyphenols are said to protect the cells against debilitating effects of oxidative stress through their ability to scavenge free radicals [33]. Phenols have antioxidant properties and may likely possess anticarcinogenic properties [34]. Steroid containing compounds are important in pharmacognosy due to their association with sex hormones [35]. There is a strong support from recent evidence on the role of polyphenols in prevention of cancers, cardiovascular diseases and osteoporosis and a suggestion of a role in prevention of diabetes mellitus and neurodegenerative diseases [33]. There is remarkable progress in the area of cardiovascular diseases, it is now confirmed that some polyphenols taken as supplements or with food improved health status shown by biomarkers related to risk of cardiovascular diseases. From literatures, there is a support of role of oxidative stress in the etiology of age-related illnesses and prevention by polyphenols from diets [33]. Primary and secondary metabolites produced by plants have many roles. The primary metabolites such as simple sugars, lipids, amino acids and nucleic acids, are needed for cellular processes. Secondary metabolites are produced in stress conditions in inhibition of herbivores [36]. Terpenes are well known as useful compound serving as precursor in the synthesis of polymers. 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 is comprised of three or six isoprene units and some studies on triterpenes showed that it can help in treating people with diabetes because it is capable of reducing 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]. In pharmacology applications, alkaloids can be used as anesthetics and CNS (Central Nervous System) stimulants [40]. Flavonoids are important group of polyohenols widely distributed among the plant flora [41]. There are health benefits in consuming foods that are rich in flavonoids once they are absorbed in the body. Xanthine oxidase and arachidonic acid metabolism can be inhibited when flavonoids act favourably in the body [42]. Flavonoids possess an anti-inflammatory activity and therefore can inhibit the production of inflammatory mediators through the modulation of arachidonic pathway, inhibiting several enzymes such as hydrolases, peroxidases, metallopeptidases, tyrosinases, ATpase, prostaglandin, cyclooxygenase, lipoxygenase, NADH Oxidase, protein kinase and phopholipases [43]. Flavonoids have antioxidant properties and also possess other physiological activities such as an anti-oxidant, anti-microbial, anti-diarrhea, anti-cancer, anti-inflammatory and anti-allergic [39]. Tannins occur naturally mostly in higher plants. Tannins are among the components that can help to reduce the risk factors associated with suffering from cardiovascular diseases and some form of cancer [44]. In medicine, plants that contain tannin can be used as astringents in treating diarrhea, as diurectics, against stomach and duodenal tumours and as anti-inflammatory, antiseptic, haemostatic pharmaceuticals [45]. Tannins can help in speeding up blood clotting processes, decreasing blood pressure, immune response modulation and reducing plasma lipid [39]. Glycosides are non-reducing organic compounds. 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 detergents or emulsifiers present in many plants. Saponins in diet have a wide activity range against fungal and bacterial infections. Other activities are in decreasing blood cholesterol levels and cancer growth inhibition. They bind with cholesterol and bile acids, eliminating fatty components out of the body and thus reducing the levels of blood cholesterol. Some also have effects on the heart and have been used for treatment of heart diseases for a long time [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 organic compounds that are important and needed in minute amounts (micronutrients). They serve basic roles in the body such as metabolism, maintenance of health and growth [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 a metal chelator, pro-oxidant, 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. Though people differ in their sensitivity to antinutrients, therefore, there is need to process food properly in order to reduce the antinutrient content of food [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. Removal of components of food that are not desirable aids in improving quality.

Various methods such as cooking, soaking and fermentation help in anti-nutritional disabling. The use of two or three of the methods mentioned above could be more efficient in eliminating antinutrients than the use of only one method [55]. Minerals may be in foods but the body must absorb them before they can be bioavailable. However, their absorption is limited by anti-nutritional constituents such as phytates and oxalates limit their absorption [56]. The minerals present in food led to the use of extrusion cooking techniques [57,58], which reduces the non-nutritive compounds that are heat-labile thereby improving the nutritive value of food [59].

The GC-MS (gas chromatography-mass spectrophotometry) of aqueous extract of fermented seeds of *Prosopis africana* recorded a total of seventeen (17) peaks which correspond to the biologically active compounds recognized by comparing the peak area (%), peak retention time, height and mass spectral fragmentation patterns to that of the known compounds as 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 various therapeutic and pharmacological actions which include anti-cancer, anti-inflammatory, anti-microbial and antioxidant. Fumaric acid is used as an acidulant and nutritional additive the farming and food industries. It is the strongest lasting food acidulant that can inhibit the growth of microorganisms, improve flavour and adjust pH. Fumaric acid is hydrophobic and this gives rise to a persisting, long-lasting sourness and flavour enhancement [60]. Fumaric acid is an organic compound that occurs naturally. It is produced in small quantities by many microorganisms and is a metabolite in the citric acid cycle. Fumaric acid has two potential new applications in addition to polymerization. Firstly, it is used in the treatment of psoriasis. Due to some biochemical defects that prevent the production of fumaric acid in the skin, individuals suffering from psoriasis are not able to produce fumaric acid in their bodies. Therefore, to treat the disease, these individuals need to orally take fumaric acid in the form of fumaric acid monoethyl or dimethyl ester. Production of fumaric acid by fermentation, many aspects that determine the productivity of the process, like the use of neutralizing agents and applied microbial strain and its morphology [61]. Siloxanes are found to be adaptable species that has so many applications as a versatile material which functions as building blocks for polymers and hybrid organic-inorganic systems. Apart from using polysiloxane in medicine, one of the most known applications of polysiloxane materials is in the manufacture of bacteria-resistance and low-fouling surfaces [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 of benefits to human body and can help in satiety and energy homeostasis through specific processes which include reducing lipogenesis level, glucose homeostasis and activation of free fatty acid receptors. Propionic acid can serve as food additive applied to produce characteristic holes and nutty flavour. Propionic acid is a strong organic acid which can be used as an anti-microbial agent in foodstuff [63]. Acetamide derivative have been found to possess analgesic activity. Anagelsics, are most widely used drugs for pain [64].

5. CONCLUSION

From the findings of this study, fermented seeds of *Prosopis africana* contained rich amount of nutrients and phytochemicals and could be a potential source of therapeutic compounds. Generally, anti-nutrients may reduce the absorption of the essential nutrients required by the body for metabolic processes. However, the small amount of the selected anti-nutrients analysed in fermented seeds of *Prosopis africana* was generally low and would not exert any health concerns.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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