Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Research Article

Evaluation of Fermented *Parkia biglobosa* (African Locust Bean) and *Bombax glabra* (Malabar Chest Nut)

Osuntokun OT*, Akele EO and Paul DA
Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba, Akoko, Ondo State, Nigeria

***Corresponding author:** Osuntokun OT, Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba, Akoko, Ondo State, Nigeria

Received: June 22, 2020; **Accepted:** July 24, 2020; **Published:** July 31, 2020

Abstract

Fermentation in food processing is the process of converting carbohydrates to alcohol or organic acids using microorganisms (yeast or bacteria) under anaerobic conditions. Fermented foods are defined as palatable products, which are prepared from raw or heated materials and acquire their characteristic properties by a process that involves microorganisms. The study was conducted to determine the fermentative activities of microorganisms on vegetable proteins of legumes or oil seeds, identify microorganisms involved in the production of food condiments and to evaluate the microbial interplay and nutritional value of fermented food condiments. Food condiments iru and fermented Malabar chest nut were produced from African locust bean (*Parkia biglobosa*) and *Bombax glabra* respectively using traditional method. Raw samples were dehulled and fermented for 120 hours. The seeds were dehulled and the hard seed coat was peeled off, the cotyledons were boiled to soften it and was tightly packed in a sack to keep the system warm and fermented for 5days to produce a fermented substrate. The organisms associated with the fermented products were identified as *Bacillus subtilis, Lactobacillus plantarium, Corynebacterium diphtheria, Actinomyces sp, Bacillus pulmilis, Lactobacillus lactis, Bacillus cereus, Rhyzopus oligosporus, Rhyzopus solonifer, Rhyzopus oryzae and Aspergillus flavus*. The pH changes occurring during the fermentation of the seeds were monitored. The pH increased proportionately with the fermentation period, ranging from 6.2 to 7.0 in African locust bean and 6.1 to 7.2 in fermented malabar chest but within 120 hours. The seeds of *Parkia biglobosa* and *Bombax glabra* were analysed for their proximate composition and phytochemical compositions. The phytochemical screening results indicates that the seeds contain Alkaloids, Flavonoids, Steroids, Terpenoids and Saponins. *Bombax glabra* lacks Tannins and glycosides which is present in *Parkia biglobosa* while *Parkia biglobosa* lacks phenols which is present in *Bombax glabra*. The proximate composition results showed that the mean nutritional content of *Parkia biglobosa* seeds contain 33.9 ± 0.05moisture content, 4.55 ± 0.02, Ash content, 5.94 ± 0.01 crude fibre, 30.5 ± 0.20 crude protein, 0.91 ± 0.02 crude fat and 24.2 ± 4.03 Carbohydrate contents. The proximate analysis also shows that the seeds of *Parkia biglobosa* and *Bombax glabra* have high moisture content, crude protein and carbohydrate content. The study showed that *Bacillus sp* are the predominant microorganisms involved in the fermentation process of leguminous seeds. The study also revealed that *Parkia biglobosa* and *Bombax glabra* contains important nutritional components and various phytochemicals like Alkaloids, Steroids, Glycosides, Saponins, Flavonoids, Terpenoids and Tannins.

Keywords: Fermented *Parkia biglobosa*; *Bombax glabra*

Introduction

The enzyme involved may be produced by microorganisms or they may be indigenous to the food or substrate (Ihekoronye and Ngoddy, 1985). fermentation results in the breakdown of complex organic substances into smaller ones through the action of catalysis. Fermentation is one of the oldest methods of food preservation known to man. In Africa, the art of fermentation is wide spread including the processing of fruit and other carbohydrate source to yield alcoholic and non-alcoholic beverages, the production of sour tasting Ogi - the fermented cereal product which provide instant energy in breakfast and convalescent diets (Adewusi et al., 1991; 1992) oil seed such as African locust bean, melon seed, castor oil seed mesquite bean and soybean are also fermented to give condiment.

The conventional substrates for these condiments production are diverse but are mainly legumes and oil seeds. Lanhouin is, however, a fish-based condiment, which is common in Benin. Lanhouin is used as ataste and flavor-enhancing condiment in some main dishes such as vegetable, slimy vegetable and tomato sauces. One condiment can be produced from more than one raw material. For instance, in Nigeria, dawadawa and iru are locally produced from three materials: African locust bean (*Parkia biglobosa*), soybean (*Glycine max*) or Bambara groundnut (*Vigna subterranea*). (Folarin and Oluwajenyo,

Citation: Osuntokun OT, Akele EO and Paul DA. Evaluation of Fermented *Parkia biglobosa* (African Locust Bean) and *Bombax glabra* (Malabar Chest Nut). J Bacteriol Mycol. 2020; 7(6): 1148.

Figure 1: Unfermented seeds of African locust bean(a) and fermented seeds of Africa locust bean (b) (iru/ dawadawa/Afitin/Sonru/soumbala).

2004) Ogiri is traditionally prepared by fermenting melon seeds (*Citrullus vulgaris*) and fluted pumpkin (*Telfairia occidentalis*) or castor oil seed (*Ricinus communis*).

Parkia biglobosa is a dicotyledonous angiosperm belonging to the family Fabaceae (Caesalpinioideae -Mimosoid clade). It is categorized under spermatophytes, vascular plants. It is a deciduous perennial that grows to between 7 and 20 metres high, in some cases up to 30 metres. The tree is a fire-resistant heliophyte characterized by a thick dark gray-brown bark. The pods of the tree, commonly referred to as locust beans, are pink in the beginning and turn dark brown when fully mature. They are 30-40 centimetres long on average, with some reaching lengths of about 45 centimetres. Each pod can contain up to 30 seeds; the seeds are embedded in a sweet, powder classification. (Thiombiano et al., 2012). kingdom: Plantae, Order: Fabales, Family: Fabaceae, Genus: Parkia, Species: *P. biglobosa*, Binomial name: *Parkia biglobosa*.

Parkia biglobosa, also known as the African locust bean (West African names: néré , dodongba, netetou, sumbala or iru) is a perennial deciduous tree of the family Fabaceae. It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust bean tree are used for medicinal purposes. As a standing tree, locust bean may have a positive effect on the yield of other nearby crops. (Ntui et al., 2012) Figure 1.

Bombax is a genus of mainly tropical trees in the mallow family. They are native to western Africa, the Indian subcontinent, Southeast Asia, and the subtropical regions of East Asia and northern Australia. It is distinguished from the genus Ceiba, which has whiter flowers

Bombax glabra grow in a large, woody, football- shaped pod, averaging 5-7 centimeters in diameter and 10-30 centimeters in length. The pod has a rough skin, five-valves, and transforms from green to brown when ripe. Inside the pod, there are several round light-brown seeds with faint white stripes. The seeds, which grow to a diameter of 1-2 centimeters, are tightly packed in rows of five in each valve and are surrounded by a soft, spongy off-white material. (Oliveira et al., 2000) Figure 2.

Bombax glabra can be consumed raw or in cooked applications such as frying, stir-frying, and roasting. They can also be ground into a flour and used to make bread. Malabar chestnuts should be soaked

Figure 2: Picture of Bombax glabra plant Source: botany.hawaii.edu.

overnight, which helps the tough skin split and peel, and then the seeds should be extracted and removed from the white, porous seed coating. *Bombax glabra* are commonly cooked in a frying pan with salt and oil or roasted in the oven. They can also be added to salads, stir-fries, eaten on their own as a snack, or ground and made into a hot drink. In addition to the nuts, the young leaves and flowers can be cooked and prepared as a vegetable and have a green, nutty flavor. Malabar chestnuts will keep for several months when stored in a cool and dark place.(Duarte et al., 2008).

Materials and Methods

Sample Collection and Preparation

Parkia biglobosa (African locust bean) and *Bombax glabra* (Malabar chest nut) used for this research were purchased from a local market in Akungba-Akoko,Ondo state, Nigeria. The fermentation process were carried out in the Department of Microbiology, faculty of Science Central laboratory, Adekunle Ajasin University, Akungba, Akoko.Ondo State,Nigeria.

Production of Fermented *Parkia Biglobosa* (African Locust Bean) 'Iru' Using Traditional Method

Raw *Parkia biglobosa* (African locust bean) bean were boiled for 6hours using a pressure pot to soften the firmly attached seed coats and further soaked in the boiling water for another 6hours. Excess water were drained off and the seeds were dehulled by slightly pounding the seeds with a large wooden mortar and pestle and further removal of the seed coat was achieved by rubbing the cotyledons between the palms of the hand and washing with water. The cotyledons were again cooked for another 3hours using a pressure pot, the hot boil water were drained off and the cotyledons were tightly packed in a sack, put in a bowl and covered with wooden trays, to keep the system warm and fermented for 5days to produce African fermented locust bean known as 'Iru'

Production of Fermented Malabar Chest Nut (*Bombax Glabra*)

The seeds were dehulled by slightly pounding with a large wooden mortar and pestle and peeling off the hard seed coat. The cotyledons were boiled for 1hour using pressure pot, the hot boiled water were drained off and the cotyledons were tightly packed in a sack, put in a bowl and covered with wooden trays, to keep the system warm and fermented for 5 days to produce fermented Malabar chest nut

Microbiological Analysis of fermented sample

The temperatures of the substrate were measured each day using a thermometer. One gram and two gram of the sample (Fermented *Parkia biglobosa* (African locust bean) and *Bombax glabra* (Malabar chest nut) were taken respectively and marched using mortar and pestle for proper homogenization. One gram of sample was diluted serially in seven folds dilution blanks and properly mixed with sterile glass rod. The 0.1ml of diluted sample was introduced into sterile plate and molten sterile agar medium (45°C) was poured. The media used were Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Peptone Water Broth (PWB). The plates were rotated gently to disperse the inoculum in medium and allowed to solidify. Then the plates were incubated at 37°C. This process was repeated for five days and the resulting colonies of microbes were subcultured to get the colonies in their pure form and to lower the density of cells. The cultures were maintained as slants on both nutrient agar and potato dextrose agar. The slants were kept refrigerated and stored for further use.

Determination of pH and Acidity of Fermented Sample

The two grams of sample taken were diluted in 20ml of distilled water; it was mixed thoroughly to form slurry. The electrode of the digital pH meter was dipped in the slurry and the pH readings were recorded after which titration were carried out by dropping two drops of phenolphthalein into diluted sample in a beaker and Sodium chloride was added till the colour turns pink indicating the level of acidity of the sample.

Examination of Culture Plate of Isolated Sample

Macroscopic: The colonial appearance of the organism was noted such as colour, shape and size after 24 hours incubation.

Characterization and Identification of Sample

Staining reaction was carried out by emulsifying one isolated 24 hour old colony in a drop of water placed at the center of a clean grease free slide passed through a Burnsen burner flame. The heat fixed smear was flooded with crystal violet for 60 seconds, after which the stain was poured off the slide and rinsed with running tap water. The smear was flooded with iodine and allowed to remain for 60 seconds which was then rinsed of with running tap water. The smear was decolorized with 95% ethanol which was immediately washed out. The smear was counter stained with safranin for 30 seconds, washed of with running tap water and then dried. The slide was then examined under oil immersion objective microscope, organism that retain the purple colour of crystal violet iodine complex was recorded as Gram positive, while those that appeared red or pink are Gram negative. Characteristics of culture were also examined and recorded as either cocci in clusters or in chains, short or long Figure 3.

Biochemical Test of Isolated Sample

Coagulase Test: Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of Staphylococcus isolates. Importantly, saurus is generally coagulase-positive, meaning that coagulase negativity usually excludes s. aureus. However it is now known that not all S. aureus are coagulase-positive (Gerard et al., 2013). It is also produced by Yersinia pestis. Coagulase reacts with prothrombin in the blood. The result complex is called staphylothrombin, which enables the

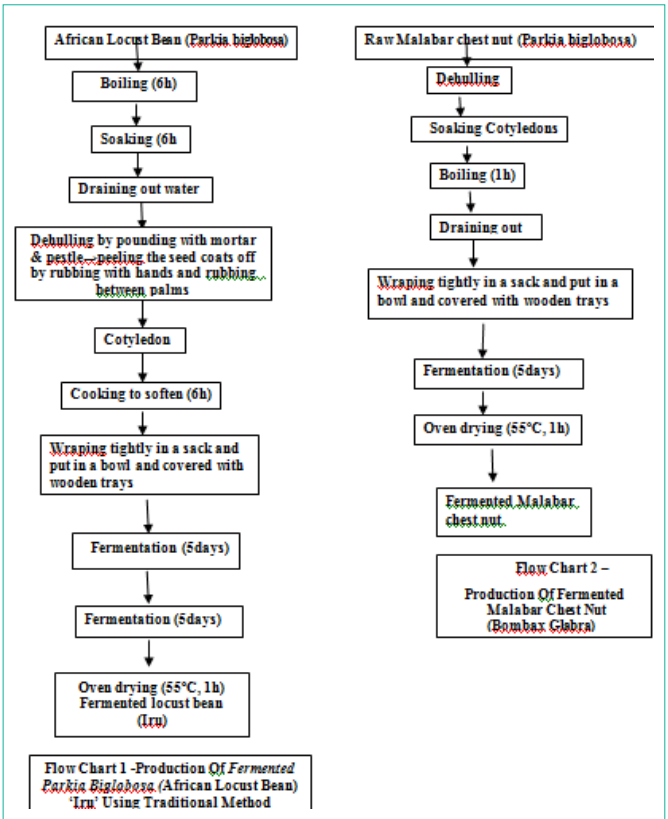

Figure 3: Characterization and Identification of Sample.

enzyme protease to convert fibrinogen, a plasma protein produced by the liver, to fibrin. This results in clotting of the blood. Coagulase is tightly bound to the surface of the bacterium S. aureus and can coat its surface with fibrin upon contact with blood. The fibrin clot may protect the bacterium from phagocytosis and isolate it from other defenses of the host. It has been proposed that fibrin-coated staphylococci resist phagocytosis, making the bacteria more virulent. Bound coagulase is part of the larger family of MSCRAMM.

The coagulase test HSS traditionally been used to differentiate Staphylococcus aureus from coagulase negative staphylococci. Saureus produces two forms of coagulase, otherwise known as "clumping factor", can be detected using a tube coagulase test. A slide coagulase test is run with a negative control to rule out autoagglutination. Two drops of saline are put on the slide is put on the slide labeled with sample number, Test (T) and control (C). The two saline drops are emulsified with the test organism using a wire loop, straight wire, or wooden stick. A drop of plasma (human plasma anticoagulated with EDTA is recommended, human serum that has been gotten from spinning fresh blood sample can also be used) is placed on the inoculated saline drop corresponding to test, and mixed well, then the slide is rocked gently for about 10bseconds. If 'positive', macroscopic clumping would be observed in the plasma within 10 seconds, with no clumping in the saline drop. If 'negative', no clumping will be observed. If the slide coagulase test is negative, a tube should follow as a confirmation. Clumping in both drops is an indication of agglutination.

This was performed for the *Staphylococcus*. The test was performed with 24 hour old culture. A loop fool of normal saline was placed on

a clean slide and a normal amount of the culture was emulsified in the normal saline to get a homogenous suspension. A drop of human plasma was added and mixed, and allowed to stand for 5 seconds. A positive reaction was observed by the formation of easily visible white clumps (Olutiola et al., 2000).

Catalase Test: Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants, and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen. (Chelikani et al., 2004). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Likewise, catalase has one of the highest turnover numbers of all enzymes;one catalase molecule can convert approximately 5million molecules of hydrogen peroxide to water and oxygen each second. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human catalase is approximately 7, and has a fairly broad maximum (the rate of reaction does not change appreciably at pH between 6.8 and 7.5). The pH optimum for other catalase varies between 4 and 11 depending on the species(Gerard et al., 2013)..

The reaction of catalase in the decomposition of hydrogen peroxide in living tissue: 2 H 2O 2->2 H 2O + O 2m The presence of catalase in a microbial or tissue sample can be tested by adding a volume of hydrogen peroxide and observing the reaction. The formation of bubbles, oxygen indicates a positive result. This easy assay, which can be seen with the naked eye, without the aid of instruments is possible because catalase has a very high specific activity, which produces a detectable response. Alternative splicing may result in different protein variants(Gerard et al., 2013).

Oxidase Test: Bacteria, which have aerobicrespiration, often have cytochrome c and a cytochrome c oxidase. The presence of these components can in combination with other methods be used for typing. A commersial test, which contains an artificial electron acceptor (N, N, N', N'- tetramethyl-p- phenylenediamine), is often used. This artificial electron acceptor change colour depending upon redox state. The substance is also referred to as a redox indicator and it can be oxidized by the oxidized form of cytochrome.

Cytochrome c oxidase is the last enzyme of the electron transport chain, where it normally reduces oxygen to water and pump protons to the outside according to the following net reaction: 4 Fe2+ -cytochrome c red + 8H +in + O 2 → 4 Fe3+ -cytochrome c ox + 2H 2O + 4H + out Cytochrome c oxidase is a transmembrane protein complex (Complex IV), which is also present in the cytoplasmic (inner) membrane of mitochondria. **Positive test result:** Dark blue-purple colour change within 10-30 sec. **Negative test result:** No colour change or colour change after more than 30 sec(Olutiola et al., 2000).

Indole test: Bacteria, which express the enzyme tryptophanase can hydrolyze the amino acid tryptophan to indole, pyruvic acid and ammonia. Presence of indole can be shown by means of Kovác's reagent or by spot indole test. In the spot test indole reacts with p-Dimethylaminocinnamaldehyde to produce a blue to blue-green product. Kovác's reagent contains p- dimethylaminobenzaldehyde, which forms a red complex with indole. **Positive Reaction:** Appearance of a blue to blue-green color change within 10 seconds. **Negative reaction:** Remain colorless or light pink.

Citrate Test: Some bacteria can utilize citrate as the only carbon source and the citrate test shows if the actual bacterium has this capability. **Negative Test Result:** No growth in citrate medium eller growth but no colour change (still green colour) in Simmon's citrate tube.

Sugar Fermentation of Isolated Sample

The carbohydrate fermentation test is used to determine whether or not bacteria can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Basal medium containing a single carbohydrate source such as Glucose, Lactose, Sucrose or any other carbohydrate is used for this purpose. A pH indicator (such as Andrade's solution, Bromcresol Purple (BCP), Bromothymol Blue (BTB) or Phenol red) is also present in the medium; which will detect the lowering of the pH of the medium due to acid production. Small inverted tubes called Durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbondioxide) (Olutiola et al., 2000).

Proximate Analysis of *Parkia Biglobosa* (African Locust Bean) and *Bombax Glabra* (Malabar Chest Nut)

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists (AOAC) methods.

Determination of Moisture Content: Determination of moisture content was done by drying samples in oven (Wise Ven, WON-50, Korea) at 110°C until constant weight was attained [Horwitz,2003].

Nitrogen Estimation: Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, KjelFlex K-360, Switzerland) method with some modification [Hussain et al,.2011].

Crude Protein: The crude protein was subsequently calculated by multiplying the nitrogen content by a factor of 6.25 [Hussain et al,.2011]. The energy value estimation was done by summing the multiplied values for crude protein.

Crude Fat and Carbohydrate: Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fat was determined by Soxhlet apparatus using n-hexane as a solvent.

Ash Value: The ash value was obtained by heating samples at 550°C in a muffle furnace (Wise Therm, FHP- 03, Korea) for 3 h [Hussain et al,.2011].

Carbohydrate Content: The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter [Hussain et al. 2011].

Crude Fiber was Estimated Crude: Fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25% NaOH (w/v) solutions [Al-Harrasi et al,.2012].

Qualitative Phytochemical Screening of *Parkia Biglobosa* (African Locust Bean) and *Bombax Glabra* (Malabar Chest Nut)

The secondary metabolite (phytochemical) screening of the

Table 1: Morphorlogical characteristics of cultured samples from fermented African locust beans.

Days	No of Colony (CFU/ML)	Size	Shape	Colour	Optical Property
Day 1	2	Med	Circular	Off White	Opaque
	6	Tiny	Irregular	Cream	Opaque
	12	Med	Circular	Pale Yellow	Opaque
Day 2	20	Med	Circular	Pale Yellow	Opaque
	11	Small	Irregular	Cream	Opaque
	22	Small	Irregular	Cream	Opaque
Day 3	25	Med	Circular	Bluish Green	Opaque
	15	Tiny	Irregular	Cream	Opaque
	19	Small	Irregular	Cream	Opaque
Day 4	30	Small	Irregular	Milk	Opaque
	35	Tiny	Irregular	Milk	Opaque
	37	Med	Circular	Cream	Opaque
Day 5	40	Med	Irregular	Bluish Green	Opaque
	32	Small	Irregular	Milk	Opaque
	42	Small	Irregular	Cream	Opaque

Table 2: Morphorlogical characteristics of cultured samples from fermented *Bombax glabra.*

Days	No of Colony (CFU/ML)	Size	Shape	Colour	Optical Property
Day 1	10	Med	Circular	Pale Yellow	Opaque
	9	Tiny	Irregular	Milk	Opaque
	3	Med	Circular	Pale Yellow	Opaque
Day 2	15	Med	Irregular	Milk	Opaque
	16	Small	Irregular	Milk	Opaque
	10	Small	Irregular	Milk	Opaque
Day 3	26	Small	Circular	Milk	Opaque
	20	Tiny	Irregular	Milk	Opaque
	24	Med	Irregular	Milk	Opaque
Day 4	40	Med	Circular	Cream	Opaque
	54	Med	Irregular	Pale Yellow	Opaque
	50	Small	Circular	Milk	Opaque
Day 5	60	Med	Irregular	Bluish Green	Opaque
	62	Small	Irregular	Bluish Green	Opaque
	64	Med	Irregular	Pale Yellow	Opaque

Key: Med: Medium

sample was carried out as described by (Osuntokun et al,.2015). The samples were screened for the following components.

Test for Saponins: To 1 ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of Saponins.

Test for Flavonoids: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange color.

Test for Alkaloids: To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates alkaloids.

Test for Glycosides: To 2 ml of plant extract, 1 ml of glacial acetic acid and 5 % ferric chloride was added. Then few drops of concentrated sulphuric acid were added. Presence of greenish blue color indicates glycosides.

Test for Terpenoids and Steroids: A fraction of the extract was dissolved in chloroform. A few drops of acetic anhydride were added followed by two drops of conc. H_2SO_4. Reddish –pink colouration indicates terpenoids and steroids.

Test for tannin: One milliliter of the filtrate were mixed with 2m1 of FeC1, A dark green colour indicated a positive test for the tannins.

Test for Phenol: Spot the extract on a filter paper. Add a drop of phoshomolybdic acid reagent and expose to ammonia vapors.Blue coloration of the spot,shows is a positive result.

Result

Table 1 and 2 shows the morphological characteristics of cultured samples from fermented African locust beans (*Parkia biglobosa*) and fermented *Bombax glabra*. The morphological characteristics include the sizes, shapes, colors and optical properties of the colony.

Table 3: Morphorlogical characteristics of isolate in Table 1.

No of days	Isolate No	Colour	Elevation	Margin	Shape
Day 1	1	Off White	Flat	Entire	Circular
	2	Cream	Convex	Entire	Circular
Day 2	1	Pale Yellow	Convex	Undulate	Circular
	3	Cream	Convex	Entire	Irregular
Day 3	1	Bluish Green	Raised	Smooth	Circular
	3	Cream	Flat	Entire	Irregular
Day 4	1	Milk	Flat	Smooth	Irregular
	3	Cream	Convex	Smooth	Circular
Day 5	1	Bluish Green	Flat	Entire	Undulate
	2	Milk	Flat	Smooth	Circular

Table 4: Morphorlogical characteristics of Isolates in Table 2.

No of days	Isolate No	Colour	Elevation	Margin	Shape
Day 1	1	Pale Yellow	Convex	Entire	Circular
	2a	Milk	Flat	Entire	Circular
	2b	Bluish Green	Flat	Entire	Underdulate
Day 2	1	Milk	Convex	Entire	Circular
	3	Milk	Convex	Entire	Circular
Day 3	1	Milk	Raised	Smooth	Irregular
	3	Milk	Flat	Entire	Irregular
Day 4	2	Pale yellow	Flat	Smooth	Circular
	3a	Milk	Convex	Undulate	Circular
	3b	Bluish Green	Flat	Smooth	Irregular
Day 5	1	Bluish Green	Raised	Smooth	Circular
	2	Bluish Green	Raised	Smooth	Circular
	3	Pale Yellow	Flat	Smooth	Irregular

Table 5: Biochemical Characteristics of Isolates.

						Sugar Fementation					
Isolate no	Gramstain	Shape	Oxi	In	Cit	Glu	Mn	Fruc	Dex	Gal	Prob. Org.
1	+	Small rod	-	-	+	AG	AG	AG	AG	AG	Bacillus subtillis
2	+	Rod	-	+	+	AG	AG	A	A	--	Lactobacillus plantarium
3	+	Tiny rod	-	-	+	A	A	A	--	--	Corynebacterium diphtheriae
4	+	Rod	-	-	+	A	A	A	A	A	Actinomyces sp
5	+	Rod	+	+	+	A	A	--	A	--	Bacillus pumilis
6	+	Rod	+	+	+	AG	AG	AG	AG	--	Lactobacillus lactis
7	+	Rod	-	-	+	A	A	--	A	--	Bacillus Cereus

Key: Fruc: Fructose; Mal: Maltose; Glu: Glucose; Mn: Mannitol; Dex: Dextrose; Gal: Galactose; Oxi: Oxidase; In: Indole; Cit: Citrate; Prob. Org.: Probable Organism; (+): Positive; (-): Negative; AG: Acid and Gas; Acid: Acid

Table 6: Morphological Characteristics of Fungal Isolates.

Isolates	Colony morphology	Microscopy view	Prob. Org.
1	Colonies were white becoming grey-brown on surface, texture deeply cottony.	Well delevoped Rhizoids situated at the point where Sporangiophores are attached to the stolons sporangiospores variable in size, average length 9-11um: very striated, elongated to polyhedric	*Rhyzopus Stolonifer*
2	Colour of Sporangiophores Appeared pale brown-brown, straight and branched with smooth walls.	Stolons are smooth, almost colourless, 5-18um in diameter- Chlamy despored are abundant c-globuse	*Rhyzopus Oryzae*
3	Stains have a large diameter (up to 43um) 8 irregular spores with most variable sizes, production of fluffy white mycelia	Large subglobose to globose spores and high proportion irregular spores (710%), spores also with nonparallel valleys and ridges and plateaus.	*Rhyzopus Oligosporus*
4	Colonies were olive green powdery with white edge spreading mycelium colonies, flat and the reverse side and green Colouration.	Conidial heads are typically radiate, later spilitting to form lose columns, biseriate conidiosphores are hyalim and coarsely roughened, often more noticeable near the vesicide	*Aspergillus Flavus*

According to the table, different sizes, shapes and colors were recorded indicating the appearance of diverse bacterial colonies.

Table 3 and 4 shows the distinct and unique colonies that are isolated from previous culture by the subculture of the former bacteria colonies. Small portion of a viable, distinct looking bacterial cell is transfered from the former culture plate to a fresh culture media. According to the table, the different colors of colonies seen are off white, cream, pale yellow, milk and bluish green while the different shapes seen are circular, irregular and undulate.

Table 5 shows the biochemical characteristics of bacterial isolates. From this table, positive rod shaped bacteria were the predominant species of bacteria found in the fermented substrates. The bacteria identified are *Bacillus subtilis, Lactobacillus planetarium, Corynebacterium diphtheria, Actinomyces sp, Bacillus pumilis, Lactobacillus lactis* and *Bacillus cereus*. According to the table, Bacillus species are the predominant species of bacteria found in both fermented seeds.

Table 6 shows the morphological characteristics of fungal isolates. From this table, based on the characteristic features that were seen in the colony morphology and in the microscopic view, the probable organisms were *Rhyzopus stolonifer, Rhyzopus oryzae, Rhyzopus oligosporus* and *Aspergillus flavus*. According to the table, Rhyzopus species are the predominant species of fungi found in the both fermented seeds.

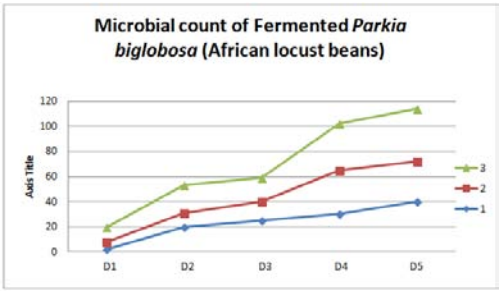

Figure 4: Microbial count of Fermented *Parkia biglobosa* (African locust beans).

Figure 4 and 5 shows the microbial count of fermented African locust beans (*Parkia biglobosa*) and fermented *Bombax glabra*. According to the table, the microbial count in fermented *Parkia biglobosa* and *Bombax glabra* increased for each day. On the last day of fermentation (D5), the highest colony count was recorded. The increase in the bacterial colony for each day indicates the activities of

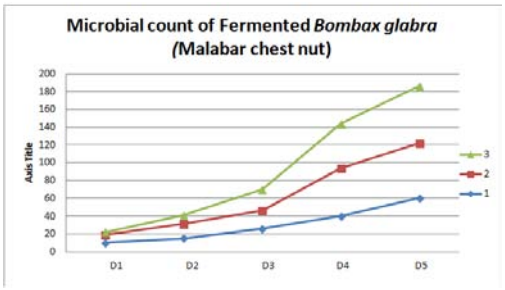

Figure 5: Microbial count of Fermented *Bombax glabra (*Malabar chest nut).

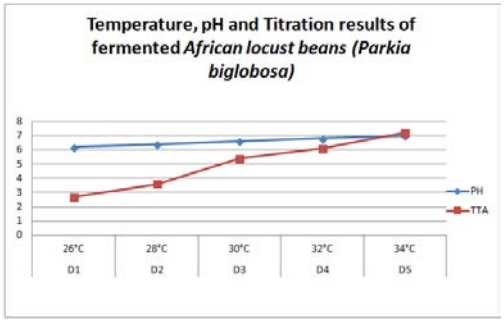

Figure 6: Temperature, pH and Titration results of fermented African locust beans (Parkia biglobosa).
Key: D: Day; TEMP: Temperature; Ph: Hydrogen ion concentration; TTA: Titration

microorganisms utilizing the substrate for their metabolic activities, growth and development and producing enzymes for fermentation.

Figure 6 shows the temperature, pH and the titration values of fermented African locust beans (*Parkia biglobosa*) for 5days. The result shown for each day indicates that the temperature, pH and acid level increases each day. The temperature was measured each day with the use of a thermometer and the pH was gotten for each day with the use of a pH meter.

Figure 7 shows the temperature, pH and the titration values of fermented *Bombax glabra* for 5days. The result shown for each day indicates that the temperature and pH increases each day while the result shown for the titration indicates that the acid level of the fermenting substrate decreases for each day. The progressive increase in the pH of fermented legumes and oil seeds compared to other materials under similar conditions have been attributed to higher protein content of the seeds. A decrease in temperature shows lower protein content in fermented *Bombax glabra*.

Table 7 shows the results for the proximate analysis of fermented *Parkia biglobosa* and fermented *Bombax glabra*. From this table, fermented *Parkia biglobosa* and *Bombax glabra* are seen to have high moisture content of 33.9±0.05 and 39.50±0.02 respectively, and ash content is low, 4.55±0.02 and 2.10±0.03 respectively. From the table also, fermented *Parkia biglobosa* and *Bombax glabra* are seen to contain high protein content of 30.5±0.20 and 27.20±0.04 while *Bombax glabra* has the highest carbohydrate content of 43.04±0.02 compared to the carbohydrate content of *Parkia biglobosa* which is 24.2±4.03.

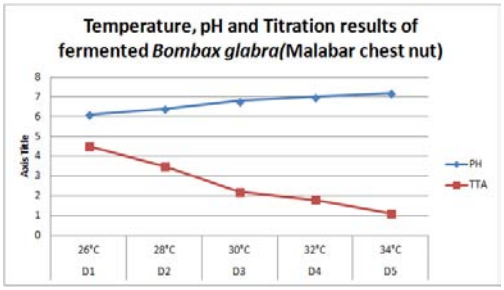

Figure 7: Temperature, pH and Titration results of fermented Bombax glabra.

Table 7: Proximate analysis of *Parkia biglobosa (*African locust bean) and *Bombax glabra (*Malabar chest nut).

Proximate Composition	Bombax	Parkia
Moisture	39.50 ± 0.02	33.9 ± 0.05
Ash	2.10 ± 0.03	4.55 ± 0.02
Fat	10.90 ± 0.02	0.91 ± 0.02
Protein	27.20 ± 0.04	30.5 ± 0.20
Crude fibre	2.20 ± 0.02	5.94 ± 0.01
Cabohydrates	43.04 ± 0.02	24.2 ±±4.03

Table 8: Qualitative Secondary metabolite (Phytochemical) screening of *Parkia biglobosa (*African locust bean) and *Bombax glabra (*Malabar chest nut).

Phytochamical Constituents	Parkia	Bombax
Alkaloids	+	+++
Flavonoids	+++	+
Tanins	++	-
Glycosides	+++	-
Steroids	++	++
Phenols	-	++
Terpenoids	+++	+++
Saponins	+++	+

Key: +: Low; ++: Minimal; +++: High; -:Abscent

Table 8 shows the result for the phytochemical screening of fermented *Parkia biglobosa* and fermented *Bombax glabra*. From this table, Alkaloid is contained in fermented *Bombax glabra* in high quantity (+++) and contained in *Parkia biglobosa* in low quantity (+). It is also seen from the table that the quantity of Terpenoid is high in both fermented legumes (+++). From the table also, *Bombax glabra* is void of Tannins and Glycosides (-) while *Parkia biglobosa* contains all Phytochemical constituents except Phenols (-).

Discussion

The purpose of this study is to determine the fermentataive activities of microorganisms on vegetable proteins of legumes and oil seeds, to identify the microorganisms involved in the production of food condiments and to evaluate the microbial interplay and nutritional value of fermented food condiments. This research work shows the mean pH values of fermentation periods. The pH values of *Parkia biglobosa* and *Bombax glabra* before fermentation were 6.2 and 6.1 respectively, after 120 hours of fermentation, the pH of *Parkia biglobosa* and *Bombax glabra* was 7.0 and 7.2 respectively. The progressive increase in pH of fermented legumes and oil seeds

compared to other materials under similar conditions have been attributed to higher protein contents of these seeds. (Achinewhu 2015).

Achinewhu (2015), reported that during the fermentation of "iru, the total of unsaturated fatty acids increased with hydrolysis of protein into amino acids and peptides. Ammonia is released due to the proteolytic activity taking place during fermentation which therefore raises the pH of the final products and giving the food a strong ammonical odour and flovour. Wang (2017) referred such fermentation as "alkaline fermentation" and this aids in prolonging shelf life of such products.

On the basics of morphological, cultural and biochemical characteristics, a total of 11 microorganisms were identified and includes seven bacterial and four fungal isolates. The bacteria isolates were identified as *Bacillus subtillis, Lactobacillus planetarium, Corynabacterium diphtheria, Actinomyces sp, Bacillus pumilis, Lactobacillus Lactis* and *Bacillus cereus* as shown in table. While fungal isolate were identified as Rhyzopus oligosporus, Rhyzopus stolonifer, Rhyzopus oryzae and *Aspergillus flavus* as shown in Table 7 and 8

Odunfa (2012) had reported that members of bacillus sp, Staphylococcus sp, Rhyzopus and Penicillin sp are the microorganisms involved in the production of iru. However, most researchers had also reported that Bacillus and Staphylococcus sp as the predominant bacteria involved in fermentations. (Baber and Achinewhu 2015; Odunfa 2012; and Sanni et al., 2002).

However, Campbell (2016) reported 83% to 93% of the total isolate in "iru' to be Bacillus sp while other organisms constituted 7% to 17% of the isolate. Also, Falegan (2017) isolated a percentage of 19.4% Bacillus sp from "iru' samples obtained from different sources. This shows that Bacillus sp is the predominant microorganism in the fermentation of African locust bean and also according to the result of isolate gotten from table 7, Bacillus sp is also the predominant microorganism in the fermentation of *Bombax glabra*.

The proximate analysis of *Bombax glabra* and *Parkia biglobosa* was presented. Proximate analysis included the moisture, ash, crude protein, crude fat, crude fibre and total carbohydrate. This analysis is important for the determination of food quality, microbial stability and can be used for nutritional labeling. The proximate analysis of fermented seeds of *Parkia biglobosa* showed percentages of constituents as thus: moisture content of 33.9±0.05, ash content of 4.55±0.02, 30.5±0.20 of total protein, crude lipid of 20.91±0.02, carbohydrates of 24.2±4.03 and crude fibre composition of 5.94±0.01 while that of *Bombax glabra*: moisture content of 39.50±0.02, ash content of 2.10±0.03, 27.20±0.04 of total protein, crude lipid of 19.90±0.02, carbohydrates of 43.04±0.02 and crude fibre composition of 2.20±0.02 as given in Table 8.

Moisture content of *Parkia biglobosa* and *Bombax glabra* was observed to be high as shown in Figure 2 and Figure 3 which to an extent gives an idea on the perishable ability nature of fruits materials due to association with the rise of microbial activities (Hassan et al., 2007; Ruzoinah et al., 2009).

The result obtained from figure 3 also showed that *Parkia biglobosa* and *Bombax glabra* seeds are high carbohydrate and oil containing seeds. A high carbohydrate food is desirable while deficiency of carbohydrate causes depletion of body tissues (Barker 2018). The major function of carbohydrate is to provide the body with energy. From the result, the consumption of fermented *Bombax glabra* will yield more energy to the body than *Parkia biglobosa*.

The protein content obtained in the *Parkia biglobosa* is high and similar to what has earlier been reported by Osuntokun et al., (2017). The observed high protein content revealed that *Parkia biglobosa* and *Bombax glabra* are good source of protein. According to the result, *Parika biglobosa* contains the highest amount of protein which is a little higher than *Bombax glabra* indicating that both seeds are rich in protein. The observed low crude fibre is in correlation to the work done by Hassan et al., (2007) which revealed the crude fibre of *Parkia biglobosa* fruit to be 4.17. Fibre plays a role in the prevention of number of diseases by reducing the level of cholesterol.

Food analysis is the resolution of the components of food into its proximate or ultimate parts (Onwuka, 2005). Proximate analysis involves the determination of the major components of food as moisture, fat, ash, protein, fiber, and carbohydrate. The sweet and fleshy product of a tree or other plant that contains seed and it can be eaten as food (Lewis, 2002).

Plants are rich in chemical constituents that have medicinal properties which help to sustain and improve human health (Soheil et al., 2013). These chemical constituents contain various phytochemical molecules which include secondary metabolites. Secondary metabolites contain the bioactive constituents of plants which are the active ingredients of many drugs. The most important of these bioactive constituents of plants are terpenes, alkaloids, flavonoids and phenolic compounds (Salem et al., 2016; Usunobun et al., 2014),(Rohit, 2015).

The phytochemical result showed the presence of alkaloids in both seeds, *Bombax glabra* showed a higher concentration (+++) of alkaloid than *Parkia biglobosa* (+). Alkaloids are known to have muscle relaxant property and can be utilized for their alalgesic, antispasmodic and bactericidal effects (Stray, 1998; and Okwu, 2004). Saponins also was detected in both seeds but of higher concentration in *Parkia biglobosa* (+++) and of low concentration in *Bombax glabra* (+). The presence of saponins in the seeds can be useful in treating inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Rita et al., 2015). Also in nature, saponins appear to act as antibiotics that protects plants from microorganisms. In humans, saponins might fight cancer and infection. (Adelani et al., 2015)

Both seeds also contain flavonoids, according to the result, *Parkia biglobosa* contains high amount of flavonoid (+++) compared to *Bombax glabra* (+). Flavonoids in plants comprise a vast array of biologically active compounds which have been used in traditional medicine for many years and have antioxidant and antiproliferative effects especially against chronic inflammatory and allergic diseases, breast cancer and coronary artery disease (Ochwang'I et al., 2016). They are also potent water-soluble antioxidants and free radical

scavengers, which prevent oxidative cell damage and have strong anticancer activity (Okwu et al., 2006).

The presence of Tannins was detected in *Parkia biglobosa* in minimal quantity (++) but absent in *Bombax glabra* (-). Tannins are astringent in taste and help in healing of wounds and inflamed mucous membrane (Njoku and Akumfula, 2007). Tannins decreases the bacterial proliferation by blocking key enzymes at microbial metabolism. Hence, the seeds could act as an efficient antimicrobial drug (Pradeepa et al., 2016). Tannins also interfere with protein synthesis.

Terpenoids were found in both seeds in high quantity. Terpenoids are antifungal and antibacterial which is attributed to their membrane disruption action and inhibitory action on bacterial cell or fungus (Tawheed and Monika, 2014).

According to the result shown in table 8, steroid is contained in both seeds in minimal proportion (++). Steroids in plants have been shown to exhibit analgesic properties and responsible for central nervous system activities (Ahmed and Mohammad, 2014)

Glycosides also was found present in *Parkia biglobosa* in high quantity but absent in *Bombax glabra*. Glycosides are beneficial in reducing inflammation, protecting against endotoxemia and may be used in cardiac treatment of congestive heart failure (Tawheed and Monik, 2014).Phenol was found present minimally in *Bombax glabra* (++) but absent in *Parkia biglobosa*. Phenols are known to be antioxidants. This means they can stop the reaction of free radicals with other molecules in the body, preventing damage to the DNA as well as long term health effects (Pradeepa et al., 2016).

The present study showed that the seed of *Parkia biglobosa* and *Bombax glabra* contain nutritional components and various phytochemicals like alkaloids, steroids, glycosides, saponins, flavonoids, terpenoids and tannins. These compounds naturally occur in most plant materials and have proven to have medicinal properties including anticancer, antitumor, antimalarial, antidiuretic, antimicrobial and antifungal activities among others. Thus, the seeds of *Parkia biglobosa* and *Bombax glabra* have potential effect on degenerative disease. The research work also revealed that *Bacillus subtilis* is the predominant microorganism involved in the fermentation of African locust bean and *Parkia biglobosa* respectively as food condiments.

Also, it is deduced from the study that fermented *Parkia biglobosa* and *Bombax glabra* contains high proportion of protein, indicating that they can be used as protein supplement and as a cheaper source of protein in food. This helps to reduce Protein-Energy-Malnutrition (PEM)). Protein-Energy-Malnutrition (PEM) is a problem confronting most nations in sub-saharan Africa (Hassan and Umar, 2005). PEM occurs due to insufficient intake of protein sources such as meat, fish and poultry products which are beyond the reach of the population due to poverty, geometric increase in population, natural disasters such as flood and desert encroachment (Hassan and Umar, 2005).

From the research work, it is seen that apart from the fact that *Bombax glabra* nut has been consumed raw, by cooking or by frying, it can also be used as food condiment by fermentation process. Almost all food products are fermentable indicating that amongst the popular substrates that have been used for fermentation (iru or dawadawa from locust bean (*Parkia biglobosa*), ogiri from melon seeds (*Citrullus vulgaris*), daddawa from soybean (*Glycine max*) etc), more substrates from other sources should be used for the production of food condiments to create multiple sources of protein and other essential nutrients that are useful for the body system.

Conclusion

The present study showed that the seed of *Parkia biglobosa* and *Bombax glabra* contain nutritional components and various secondary metabolite(phytochemicals) like alkaloids, steroids, glycosides, saponins, flavonoids, terpenoids and tannins. These compounds naturally occur in most medicinal plants and have proven to have medicinal properties including anticancer, antitumor, antimalarial, antidiuretic, antimicrobial and antifungal activities among others. Thus, the seeds of *Parkia biglobosa* and *Bombax glabra* have potential effect on degenerative disease. It was also revealed that Bacillus subtilis is the most predominant microorganism involved in the fermentation of African locust bean(*Parkia biglobosa*) and fermented *Parkia biglobosa* and *Bombax glabra* contains high proportion of protein, indicating that they can be used as protein supplement and as a cheaper source of protein in food. This helps to reduce Protein-Energy-Malnutrition (PEM)). Protein-Energy-Malnutrition (PEM) is a problem confronting most nations in sub-saharan Africa.

References

1. Achinewhu SC. Chemical and nutrient composition of fermented products from plant foods Nig.Fd. . J. 2015; 1: 115-116.
2. Achinewhu SC, Baber LI, Jeoma IO. Physicochemical Properties and Garifcation (Gari Yield) of Selected Cassava Cultivars in Rivers State Nigeria. Plant Food Human Nutr. 1998; 52: 133.
3. Adelani A, Tabitha A, AjibaLilian C, Dahunsi SO, Oluyori AP. Antibacterial activity of watermelon (Citrullus lanatus) seed against selected microorganisms. . Afr. J. Biotechnol. 2015; 14: 1224-1229.
4. Adewusi SRA, Orisadare BO, Oke OL. Studies on wearing Diet in nigeria, 1. Carbohydrate source. Cereal chemistry. 1991; 165-169
5. Adewusi SRA, Orisadare BO, Oke OL. Studies on wearing Diet in nigeria, 2. Protein source. Plant food for Human Nutrition. 1992; 42: 183-192.
6. Ahmed O, Mohammad Ali. Qualitative and Quantitative Analysis of Phytochemicals of Loranthus bengwensi leaf. Int. R. J. Pharm. Sci. 2014; 05: 1-3.
7. Aiyelegun T, Owolarafe O, Ogunsina B, Taiwo S. Inconsistency in the Physical Properties of Gari Produced in Southern Nigeria.: A Proposal for Appropriate Technology. Journal of Process Engineering. 2017.
8. Al-Harrasi A, Al-Rawahi A, Hussain J, Rehman N, Ali L, Hussain H. Proximate analysis of the resins and leaves of Boswellia sacra. J Med Plants Res. 2012; 6: 3098-3104.
9. Barker. The effect of fermentation on carbohydrate and fatty acid composition of African oil bean seed (*Pentacletra macrophylla*). Food Chem. 2018; 19: 105-116.
10. Campbell. Microorganisms associated with fermentation of African Locust Bean during preparation. J. Plant Foods. 2016; 245-250.
11. Cannell JJ, Hollis BW, Zasloff M, Heaney RP. Diagnosis and treatment of vitamin D deficienc..ExpertOpin Pharmacother. 2008; 9: 107-18.
12. Chelikani P, Donald LJ, Duckworth HW, Loewen PC. Hydroperoxidase II of Escheriochia coli exhibits enhanced resistance to proteolytic cleavage compared to other catalases. Biochemistry. 2013; 42: 5729-5735.
13. Duarte, Odilo; Robert E. Paull. Pachiraaquatica: Malabar chestnut". In Jules

Janick, Robert Paull (ed.)." The Encyclopedia of Fruit and Nuts Cambridge, MA: CABI. 2006; 182-183.

14. Falegan OS, Ball MW, Shaykhutdinov RA, Pieroraio PM, Farshidfar F, Vogel HJ, Allaf ME, Hyndman M.E(2017). Urine and Serum Metabolomics Analyses May Distinguish between Stages of Renal Cell Carcinoma. Metabolites. 2017; 7: 6.

15. Folarin A, Oluwajenyo MO. Incidence and characterization of Bacillus cereus isolated from traditional fermented meals in Nigeria. J Food Prot. 2004; 67: 2805-8.

16. Gernah DI, Inyang CU, Ezeora NL. Incubation and fermentation of African locust beans (Parkia biglobosa) in production of"dawadawa". Journal of Food Processing andPreservation. 2007; 31: 227-239. .

17. Hassan LG, Umar KJ. Protein and Amino acid composition of African Locust Bean (Parkia biglobosa)Tropical and Subtropical Agroecosystems. 2005; 5: 45-50.

18. Hassan O, El-Mahmood AM, Amech DM. In vitro Antibacterial activity of Parkia biglobosa root, Bark extract against some microorganism. Afr. J. Biotechnol. 5: 1371 1257.

19. Heuzé V, Thiollet H, Tran G, Edouard N, Lebas F. African locust bean (Parkia biglobosa &Parkia filicoidea). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. 2018; 13: 49.

20. Horwitz W, Editor. Official methods of analysis of AOAC International; 17th ed. USA: Association of Official Analytical Communities. 2006.

21. Hussain J, Rehman N, Al-Harrasi A, Ali L, Ullah R, Mabood F. Nutritional prospects and mineral compositions of selected vegetables from Dhoda Sharif Kohat. J Med Plants Res. 2011; 5: 6509-6514.

22. Ihekoronye AI. Integrated Food Science and Technology for the Tropics. Macmillian Publishers, London. 1987; 123-127.

23. Lewis R. CRC Dictionary of Agricultural Sciences, CRC Press. 2004; 4.

24. Njoku HO, Ogbulie JN, Nnubia C. African oil bean (Pentaclethra macorphylla Bentham) for ugba production. Journal of Food Science. 1990; 49: 13-26.

25. Ntui VO, Uyoh EA, Urua IS, Ogbu U, Okpako EC. Regeneration of Parkia biglobosa Benth.: An important tree species of Africa. Journal of Microbiology and Biotechnology. Research. 2012; 2: 169-177.

26. Ochwang'I DO, Kimwele CN, Oduma JA, Gathumbi PK & Kiama SG. Phytochemical screening of medicinal plants of the Kakamega Country, Kenya commonly used against Cancer. . Med Aromat Plants. 2016; 5: 277.

27. Odunfa SA. African fermented foods, In: Wood, B.J.B (ed.) Microbiology of Fermented Foods, (Vols. vol. 11). Amsterdam: Elsevier Applied Science Publishers. 1987; 155-191.

28. Odunfa SA, Daddawa, In: Reddy, Pierson NR, and Salunkhe MD, DK. (ed). Legume-based Fermented Foods. Boca Raton, CRC Press Inc. 2012; 173-189.

29. Okwu D. Phytochemicals and vitamin content of indigenous species of south-eastern Nigeria. J. Sustain. Agric. Environ. 2004; 6: 30-37.

30. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. Afr. J. Biotech. 2006; 5: 357-361.

31. Oliveira JTA, Vasconcelos IM, Bezerra LCNM, Silveira SB, Monteiro ACO, Moreira RA. Composition and nutritional properties of seeds from Pachira aquatica Aubl, Sterculia striataSt Hil et alud and Terminalia catappa Linn In Food Chemistry. 2000; 70: 185-191.

32. Olutiola PO, Famurewa O, Sonntag HG. Introduction to General Microbiology: A Practical Approach. 2nd Edn., Bolabay Publications, Ikeja, Nigeria. 2000.

33. Onwuka G. Food analysis and instrumentation, proximate composition of food minerals 1stEdition. Naplithali print, a Division of H.G. support Nigerian Ltd, Nigeria. 2005; 114.

34. Osuntokun OT, Jemilaiye TA, Akinrodoye AR. Comparative study between the effect of Parkia Biglobosa (JACQ) benth and conventional antibiotics against multiple antibiotic resistant Uropathogenic bacteria (MARUB). MOJ Bioequiv Availab. 2018; 5: 200-212.

35. Osuntokun OT, Olajubu FA. Antibacteria and phytochemical properties of some Nigeria medicinal plants on Salmonella typhi and Salmonella paratyphi isolated from human stool in Owo Local Government, Ondo State, Nigeria. Journal of Scientific Research and Reports. 2015; 4: 441-449.

36. Pradeepa M, Kalidas V, Geetha N. Qualitative and quantitative phytochemical analysis and bactericidal activity of pelargonium graveolens l'her. Int J App Pharm. 2016; 8: 7-11

37. Rita N, Baruah KK, Sarma S, Bhuyan R, Roy DC, Mithu D. Phytochemical Screening of Different Plants of North-Eastern Region of India. J Biosci Bioeng. 2015; 2: 9-11.

38. Ruzoinah U, Dacie DV, Lewis SM. Practical Haematology, 4th edition, . Longman Group Ltd, Hong Kong. 2007; 17-22.

39. Salem ME, Atega A, Afifa O, Fouzy A. Qualitative and quantities analysis of phytochemicals of various extract for Ephedraaltissima from Libya. J. Med. Plants Stud. 2016; 4: 119-121.

40. Sanni AI, Ayenor GS, Sakyi-Dawson E, Sefa-Dedeh S. Aerobic spore forming bacteria and chemical composition of some Nigerian fermented soup condiments. . Plant Foods for Human Nutrition. 2000; 55: 111-118

41. Soheil ZM, Bey HG, Chim KC, Tara S, Habsah AK. Biological activities and phytochemicals of Swietenia macrophylla King. Molecules. 2013; 18: 10465-10483.

42. Tawheed A, Monika T. A comparative study on proximate composition, phytochemical screening, antioxidant and antimicrobial activities of Linum usitatisimum L. (flaxseeds). Int. J. Curr. Microbiol. App. Sci. 2014; 3: 465-481.

43. Thiombiano DN, Lamien N, Dibong DS, Boussim IJ, Belem B. The role of woody species in managing food shortage in Burkina Faso. Sécheresse. 2012; 23: 86-93.

44. Usunobun U, Okolie NP, Anyanwu OG, Adegbegi AJ. Phytochemical screening and proximate composition of Annona muricata leaves. EJBPSP. 2014; 2: 18-28.

45. Wang. The effect of fermentation on carbohydrate and fatty acid composition of African oil bean seed (*Pentacletra macrophylla*). Food Chem. 2017; 16: 105-116.