




# In-vitro antibacterial activities of fermented and unfermented *Parkia biglobosa* seeds against selected entero-pathogens

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## Abstract

The study examined the antibacterial effects of both fermented and unfermented ethanolic extracts of *Parkia biglobosa* seeds against selected entero-pathogenic bacteria. The antibacterial activity was evaluated using agar well diffusion to determine the effect of the extracts against selected entero-pathogenic bacteria; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli*, *Citrobacter youngae*, *Klebsiella oxytoca* and *Acinetobacter haemolyticus*. Antioxidant activities of the extracts were quantified by measuring its total flavonoid and total phenol contents, 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid and ferric reducing antioxidant power. Phytochemical screening and Gas Chromatography–Mass Spectrometry were carried out on both samples using standard methods. The sensitivity test revealed that *E. coli*, *S. aureus*, *C. youngae*, *A. haemolyticus* and *K. oxytoca* were more susceptible (25.0 mm, 24.0 mm, 18.0 mm, 27.0 mm, and 17.0 mm) at 100 mg/ml to the crude ethanolic extract of fermented seeds, while *S. aureus*, *E. coli*, and *A. haemolyticus* were more susceptible to the unfermented extract. The seed extracts of fermented and unfermented *P. biglobosa* were found to be high in phytochemicals such as alkaloids, flavonoids, cardiac, steroids, glycosides, saponins, and tannins. Results showed that calcium, magnesium, sodium, potassium, zinc, iron, phosphorus, manganese, and copper were present in fermented and unfermented samples. The findings indicated that *P. biglobosa* seeds had considerably high and dose-dependent DPPH radical scavenging and ferric-reducing properties comparable with respective standards. The various constituents of *P. biglobosa* extracts enhanced its antibacterial activities. This study concluded that *P. biglobosa* can be a potential source of bioactive compounds and antioxidants.

**Keywords** *Parkia biglobosa* · Fermentation · Entero-pathogens · Antibacterial · Proximate · And antioxidants

## Introduction

Plessas (2022) maintained that fermentation is a production of low food processing and storage method that has been used for centuries to incorporate the transformation and energy metabolism actions of various beneficial microbes, resulting in the bioconversion of fresh produce. Fermentation is a method that uses microorganisms to turn large natural molecules into smaller molecules. Yeast enzymes, for example, turn sugars and starches into alcohol, whereas proteins are turned into peptides/amino acids. Microbes or metabolic enzymes' actions on food products often seem to ferment food, resulting in preferable chemical reactions that are responsible for substantial food alteration (Sharma et al. 2020).

Food plants commonly eaten by man has long been used as the source of medicine to cure various ailment in the human and animal population respectively, this is

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due to their various constituent compounds which elicit antimicrobial properties against most microbial infections (Akinyemi et al. 2005).

*Parkia biglobosa*, also known as the African locust bean (West African names: dodongba, netetou, néré, iru, or sumbala), is a Fabaceae family perennial deciduous tree. It grows in a variety of surroundings throughout Africa and is mainly cultivated for its shells, which have lovely tissue and vital seeds. Fermenting and crushing these seeds is a significant financial exercise in the areas where the tree is grown (Osuntokun et al. 2020). *P. biglobosa* has been widely used as a source of food and natural medicine in West African countries for centuries. The bark, roots, leaves, flowers, fruits, and seeds are commonly used in traditional medicine to treat a wide variety of complaints, both internally and externally, and are sometimes combined with other medicinal plants (Muhammed et al. 2021). Medicinal applications include therapies for parasitic infections, circulatory system disorders such as arterial hypertension, and respiratory, digestive, and skin disorders (Ayo-Lawal et al. 2014).

*Parkia biglobosa* is a plant with chemotherapeutic potential; a variety of medicinal applications for this plant have been reported (Abioye et al. 2013). Traditional uncontrolled fermentation of seeds produces commonly fermented *P. biglobosa* seeds, which are used as a condiment of food in countries in Africa such as Nigeria Burkina Faso, Benin, Senegal, and Ghana among others (Camara et al. 2016; Nitiema-Yefanova et al. 2020). Unfermented seeds had more alkaloids, tannins, and phytate than fermented seeds, which contained more flavonoids and saponins (Okwunodulu et al. 2021).

Herbs and spices' antimicrobial activity is determined by the kind of essential oil, the kind of food, and the type of microorganisms (Gutierrez et al. 2008). The chemical structure of essential oils derived from herbs and spices determines their effectiveness, specifically the presence of hydrophilic functional groups such as hydroxyl groups (Muhammad and Syeda 2017).

Enteropathogens are organisms that can cause diseases in the gastrointestinal tract (Parekh and Chanda 2007). A healthy gut microbiome requires a delicate balance of microbial groups. Microbiota deviations have been linked to an increased risk of specific diseases such as inflammatory bowel disease, irritable bowel disease, antibiotic-associated diarrhea, and diabetes (Adetutu et al. 2011). Entero-pathogenic infections are fast becoming difficult to treat due to the development of resistance to various antibiotics used in their treatment. To develop antimicrobial agents, efforts have been made from natural sources to achieve better chemotherapeutic effects with fewer side effects. The antibacterial potentials of *P. biglobosa* against enteropathogenic bacteria, the proximate, mineral, and phytochemical composition, as well

as the antioxidant potentials of unfermented and fermented *P. biglobosa* seeds, were investigated in this study.

## Materials and methods

### Collection and authentication of samples

*Parkia biglobosa* seeds were obtained from the open market in Ikare-Akoko, Akoko North East Local Government of Ondo State (7° 31' 0" N, 5° 45' 0" E). The plant was identified and authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Nigeria. The seeds were taken to Adekunle Ajasin University's Microbiology Laboratory in Akungba-Akoko for analysis.

### Test organisms

The pathogenic bacteria used were those known to cause intestinal tract diseases in humans. Eight bacterial isolates were obtained from the culture's stock of organisms at the Adekunle Ajasin University Health Center, Akungba-Akoko. The organisms utilized in the tests were standard strains of pathogenic enteric bacterial isolates. They include *Staphylococcus aureus* ATCC 25923, *S. haemolyticus* ATCC 29970, *Escherichia coli* ATCC 25922, *Citrobacter youngae* ATCC 29220, *Kleibseila oxytoca*, *Acinetobacter haemolyticus*, *Acinetobacter baumannii* ATCC 17978, and *Pseudomonas auriginosa* as they were identified via API staph and microbat™ 24E identification kits.

### Standardization of test organisms

McFarland standards of 0.5 were used to standardize all organisms. A 0.2 ml aliquot of 24 h broth culture was dispensed into another sterilized 20 ml Mueller–Hinton broth and incubated for 3 to 5 h. 1 ml of the finished broth contained 0.5 McFarland standards ( $6 \times 10^8$  CFU/ml) (Oyeleke et al. 2008).

### Preparation of locust bean seeds for fermentation

Following the method of Achi (2005), *P. biglobosa* seeds were prepared for fermentation. Three kilograms of the locust bean seeds were measured and foreign particles inside were handpicked and washed to remove impurities before cooking. Raw *P. biglobosa* seeds were cooked for 4–6 h using a pressure pot to soften the seed's coat. The cooked bean seeds were dehulled mechanically by pressing them softly with the help of a pestle and mortar. The dehulled bean seeds were washed in water using a local basket to remove fragmented seeds' coats and dirt. The cotyledons were divided into two parts of 3 g each; the first part was

sun-dried and milled into powder and stored before use and the second part was boiled in retort for 1 hour using a pressure pot, drained, and cooled to 40 °C. The hot seeds were spread in wide calabash trays (10 cm deep) which were pre-stacked with an absorbent sheet (sterile polythene sheet). Trays were stacked together and wrapped with jute bags after which it was incubated (35 °C) for 36 h for natural fermentation. The fermented bean seeds were dried using sunlight for 3 days and milled into powder and packed for analysis.

### Preparation of plant extracts

Five hundred grams of fine powdered fermented and unfermented *P. biglobosa* seeds were weighed into corked bottles containing 1000 ml of absolute ethanol each, and the mixture was vigorously shaken daily for 7 days. The filtrates were collected directly into sterile crucibles after the mixtures were filtered using filter papers. The filtrates were extracted using a Soxhlet extractor, and the residues were stored at – 4 °C. The unfermented sample was treated in the same way (Musa et al. 2016).

### Antimicrobial screening of the solid extracts

The method of agar well diffusion was used to screen *P. biglobosa* seed extracts for antibacterial properties against enteropathogenic isolates. To make a stock concentration of 100 mg/ml, 1 g of each extract was dissolved in 10 ml of Dimethyl sulfoxide (DMSO) diluted with sterile distilled water in a 1:3 ratio. The filtrates were then concentrated into 50 mg/ml, 100 mg/ml, and 25 mg/ml using a dilution formula ( $C_1V_1 = C_2V_2$ ). One milliliter of 4–10 normal saline dilution of 24 h broth culture was mixed with 19 ml of agar in a sterilized universal bottle and allowed to flow into a sterilized petri dish. Before boring wells into the agar plate with a 6 mm cork borer, it was allowed to settle. Each well received 50 µl of each extract concentration and was incubated at 37 °C for 24 h. The diameters of the zones of inhibition were measured and recorded in millimeters, and the results were interpreted using Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI 2016). As a control, 0.125 mg/ml tetracycline was used.

### Mineral determination and proximate composition

According to AOAC (2005), the sample composition of proximate was determined, which included the moisture content, ash content, crude fiber, crude protein, crude fat, and carbohydrate. An atomic absorption spectrophotometer was used to determine the iron, calcium, potassium, sodium, and magnesium contents of the samples (Perkin Elmer 500 and Varian AA 475). The concentration of each element was calculated and expressed as percentage on dry matter basis.

### Quantitative phytochemical analysis

The concentration of flavonoids in the extract was estimated spectro-photometrically according to the procedure of Sun et al. (1998). The estimation of cardiac glucosides (Born-trager's Test) was determined using the method described by Fabricant and Farnsworth (2001). The aqueous extract was screened for the presence of tannin, saponin, steroid, phlobatanin, terpenoid, alkaloid, and anthraquinone was determined quantitatively by the methods described by Daramola (2015).

### Antioxidant level determination of *P. biglobosa* seeds

The antioxidant level of the unfermented and fermented seeds of *P. biglobosa* was determined using various Spectrometric techniques of AOAC (2016) which rely on the reaction of a radical and complex with an antioxidant molecule capable to donate a hydrogen atom. The antioxidant parameters (Total Phenol, Total flavonoid, ferric reducing property, free radical scavenging ability, NO radical scavenging ability, Fe<sup>2+</sup> Chelation, ABTS scavenging ability and Superoxide anion scavenging activity assay) were determined using the following procedures: the total phenol content of the extract was determined by the method of Singleton et al. (1999). 0.2 ml of the extract was mixed with 2.5 ml of 10% Folin ciocalteau's reagent and 2 ml of 7.5% Sodium carbonate. The reaction mixture was subsequently incubated at 45 °C for 40 min, and the absorbance was measured at 700 nm in the spectrophotometer, gallic acid was used as standard. The total flavonoid content of the extract was determined using a colorimeter assay developed by Bao et al. (2005). 0.2 ml of the extract was added to 0.3 ml of 5% NaNO<sub>3</sub> at zero time. After 5 min, 0.6 ml of 10% AlCl<sub>3</sub> was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rating equivalent.

The reducing property of the extract was determined as described by Pulido et al. (2002), 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50 °C for 20 min., thereafter 0.25 ml of 10% TCA was also added and centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1% of FeCl<sub>3</sub> and the absorbance was measure at 700 nm. The free radical scavenging ability of the extract against DPPH (1, 1-diphenyl-2-picrylhydrazyl) using Gyamfi et al. (1999) method. One milliliter of the extract was mixed with 1 ml of the 0.4 mM methanolic solution of the DPPH, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. The Sodium Nitroprusside in aqueous

solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Briefly, 5 mM sodium nitroprusside in phosphate- saline was mixed with the extract, before incubation at 25 °C for 150 min. Thereafter the reaction mixture was added to the Greiss reagent. Before measuring the absorbance at 546 nm, relative to the absorbance of a standard solution of potassium nitrate treated in the same way with Greiss reagent (Kumar et al. 2012).

The ability of the extract to chelate Fe<sup>2+</sup> was determined using a method of Minotti and Aust (1987) modified by Puntel et al. (2005). Briefly, 150 mM FeSO<sub>4</sub> was added to a reaction mixture containing 168 ml of 0.1 M Tris–HCl pH 7.4, 218 ml saline, and after extraction the volume was made up with 1 ml with distilled water. The reaction mixture was incubated for 5 min, before the addition of 13 ml of 1, 10-phenanthroline and then absorbance read at 510 nm. The 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability of the extract was determined according to the method described by Re et al. (1999). The ABTS was generated by reacting a (7 mM). ABTS aqueous solution with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (2.45 mM/l, final conc.) in the dark for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol 0.2 of the appropriate dilution of the extract was then added to 2.0 ml of ABTS solution and the absorbance was read at 732 nm after 15 min. The TROLOX equivalent antioxidant capacity was subsequently calculated.

Furthermore, the superoxide anion radicals were produced in 2 ml of phosphate buffer (100 mM, pH 7.4) with 78 µM β- nicotinamide adenine dinucleotide (NADH), 50 µM nitro blue tetrazolium chloride (NBT) and test samples at different concentrations. The reaction mixture was kept for incubation at room temperature for 5 min. and added with 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT was read at 560 nm. Gallic acid was used as a positive control agent for comparative analysis (Kumar et al. 2012).

### Gas chromatography and mass spectroscopy (GCMS)

The GC–MS analysis of ethanolic and ethyl acetate extract of fermented and unfermented N-hexane, acetone, and ethanol-extracted oil of *P. biglobosa* seeds was performed using the method of Oladipupo et al. (2013), a GC Clarus 500 Perkin Elmer system, with an AOC-201 auto-sampler and as chromatograph interfaced to a mass spectrometer instrument. The column used was an Elite 1 fused silica capillary column, operating in electron impact mode at 70 eV. Helium

was used as the carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µl was employed. The injector temperature was set at 250 °C and the ion-source temperature at 280 °C. The oven temperature was programmed from 110 to 280 °C, with an increase of 10 °C/min, and then held at 280 °C for 9 min. Mass spectra were taken at 70 eV and a scanning interval of 0.5, with fragments from 40 to 550 Da.

### Statistical analysis

Statistical significance was determined using ordinary one-way analysis of variance (ANOVA) and two-way ANOVA, while multiple comparisons between means were made by Tukey's or Sidak's multiple comparisons test. Analysis was performed using GraphPad Prism software (GraphPad Software Inc. La Jolla, CA, USA). All data are expressed as means of triplicates ± SEM or SD and values of p < 0.05 were considered significant, where “n” represents independent experiments.

## Results and discussions

### Sensitivity screening of crude ethanol extracts of fermented and unfermented *Parkia biglobosa* seeds on clinical isolates

Results presented in Table 1 show the zones of inhibition of fermented and unfermented crude ethanol extract of *P. biglobosa* seeds on entero-pathogenic bacteria at varying concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml respectively with *A. haemolyticus* and *E. coli* having zones of inhibition of 27.0 mm and 25.0 mm at 100 mg/ml while *C. youngae* still has a 3.0 mm inhibition at a lower concentration of 12.5 mg/ml for fermented extracts while Gram-negative organisms i.e. *E. coli* and *K. oxytoca* show more sensitivity to the extracts with clear zones of 28.0 mm and 20.0 mm respectively at 100 mg/ml to the fermented extract although the organisms are sensitive to the extract at the lowest concentration of 12.5 mg/ml for fermented and unfermented extracts. The minimum inhibitory concentration observed in *P. aeruginosa* for the fermented sample was high as compared to the unfermented sample for both solvents. However, Komolafe et al. (2014) reported a MIC of 3.125 mg/ml for stem bark extract against *Shigella* sp. and Millogo-Kone et al. (2007) reported a MIC of 0.315 mg/ml for *P. biglobosa* stem bark extract, both of which are within the range of the MIC observed in both extracts.

**Table 1** Sensitivity screening of crude ethanol extracts of fermented and unfermented *Parkia biglobosa* seeds on clinical isolates (mm)

Microorganisms	Concentrations of extract (mg/ml)														
	100			50			25			12.5			(Tetracycline 30 µg)		
	UFE	FEE	UFE	FEE	UFE	FEE	UFE	FEE	UFE	FEE	UFE	FEE	UFE-C	FEE-C	
<i>Acinetobacter baumannii</i>	25.0 ± 0.7 <sup>c</sup>	23.0 ± 0.02 <sup>c</sup>	19.0 ± 0.5 <sup>b</sup>	19.0 ± 0.3 <sup>c</sup>	15.0 ± 0.1 <sup>c</sup>	10.0 ± 0.12 <sup>b</sup>	6.0 ± 0.3 <sup>b</sup>	5.0 ± 0.0 <sup>b</sup>	30.0 ± 0.1 <sup>e</sup>	28.0 ± 1.0 <sup>cd</sup>					
<i>Staphylococcus haemolyticus</i>	18.0 ± 0.3 <sup>a</sup>	13.0 ± 0.21 <sup>a</sup>	16.0 ± 0.2 <sup>a</sup>	11.0 ± 0.7 <sup>a</sup>	9.0 ± 0.5 <sup>a</sup>	8.0 ± 0.5 <sup>ab</sup>	5.0 ± 0.5 <sup>ab</sup>	3.0 ± 0.5 <sup>a</sup>	23.0 ± 0.5 <sup>b</sup>	26.0 ± 0.5 <sup>c</sup>					
<i>Escherichia coli</i>	27.0 ± 0.52 <sup>c</sup>	25.0 ± 0.5 <sup>cd</sup>	22.0 ± 0.6 <sup>c</sup>	18.0 ± 0.1 <sup>b</sup>	18.0 ± 0.7 <sup>c</sup>	12.0 ± 0.02 <sup>c</sup>	8.0 ± 0.5 <sup>ab</sup>	6.0 ± 0.5 <sup>b</sup>	29.0 ± 0.5 <sup>d</sup>	20.0 ± 0.4 <sup>a</sup>					
<i>Citrobacter youngae</i>	20.0 ± 0.12 <sup>ab</sup>	18.0 ± 0.4 <sup>b</sup>	19.0 ± 0.5 <sup>b</sup>	17.0 ± 0.9 <sup>b</sup>	12.0 ± 0.5 <sup>b</sup>	11.0 ± 0.5 <sup>c</sup>	3.0 ± 0.5 <sup>a</sup>	5.0 ± 0.5 <sup>b</sup>	25.0 ± 0.6 <sup>bc</sup>	23.0 ± 0.5 <sup>b</sup>					
<i>Klebsiella Oxytoca</i>	20.0 ± 0.08 <sup>ab</sup>	17.0 ± 0.7 <sup>ab</sup>	18.0 ± 0.3 <sup>b</sup>	16.5 ± 0.5 <sup>b</sup>	15.0 ± 0.3 <sup>c</sup>	12.0 ± 0.3 <sup>c</sup>	5.0 ± 0.5 <sup>ab</sup>	4.0 ± 0.5 <sup>ab</sup>	20.0 ± 0.5 <sup>a</sup>	20.0 ± 0.2 <sup>a</sup>					
<i>Acinetobacter haemolyticus</i>	27.0 ± 0.05 <sup>d</sup>	27.0 ± 0.05 <sup>d</sup>	23.0 ± 0.2 <sup>c</sup>	19.0 ± 0.8 <sup>c</sup>	10.0 ± 0.5 <sup>a</sup>	10.0 ± 0.3 <sup>ab</sup>	5.0 ± 0.5 <sup>ab</sup>	5.0 ± 0.5 <sup>b</sup>	28.0 ± 0.4 <sup>d</sup>	24.0 ± 0.5 <sup>b</sup>					
<i>Staphylococcus aureus</i>	28.0 ± 0.03 <sup>d</sup>	24.0 ± 0.07 <sup>c</sup>	24.0 ± 0.3 <sup>c</sup>	20.0 ± 0.3 <sup>c</sup>	15.0 ± 0.3 <sup>c</sup>	10.0 ± 0.7 <sup>b</sup>	6.0 ± 0.5 <sup>b</sup>	4.0 ± 0.5 <sup>ab</sup>	30.0 ± 0.5 <sup>e</sup>	22.0 ± 0.1 <sup>ab</sup>					
<i>Pseudomonas aeruginosa</i>	25.0 ± 0.05 <sup>e</sup>	23.0 ± 0.3 <sup>c</sup>	19.0 ± 0.1 <sup>b</sup>	13.0 ± 0.4 <sup>a</sup>	15.0 ± 0.6 <sup>c</sup>	6.0 ± 0.4 <sup>a</sup>	6.0 ± 0.5 <sup>b</sup>	0.0 ± 0.0	28.0 ± 0.5 <sup>d</sup>	21.0 ± 0.5 <sup>a</sup>					

The values are the SD of the means of three replicates. Values with the same superscript down the column are not significantly different; however, at  $P < 0.05$ , a significant difference exists

Different superscripts indicate significant difference

FEE fermented ethanol extract, UFE unfermented ethanol extract

## Quantitative phytochemical analysis of fermented and unfermented *Parkia biglobosa*

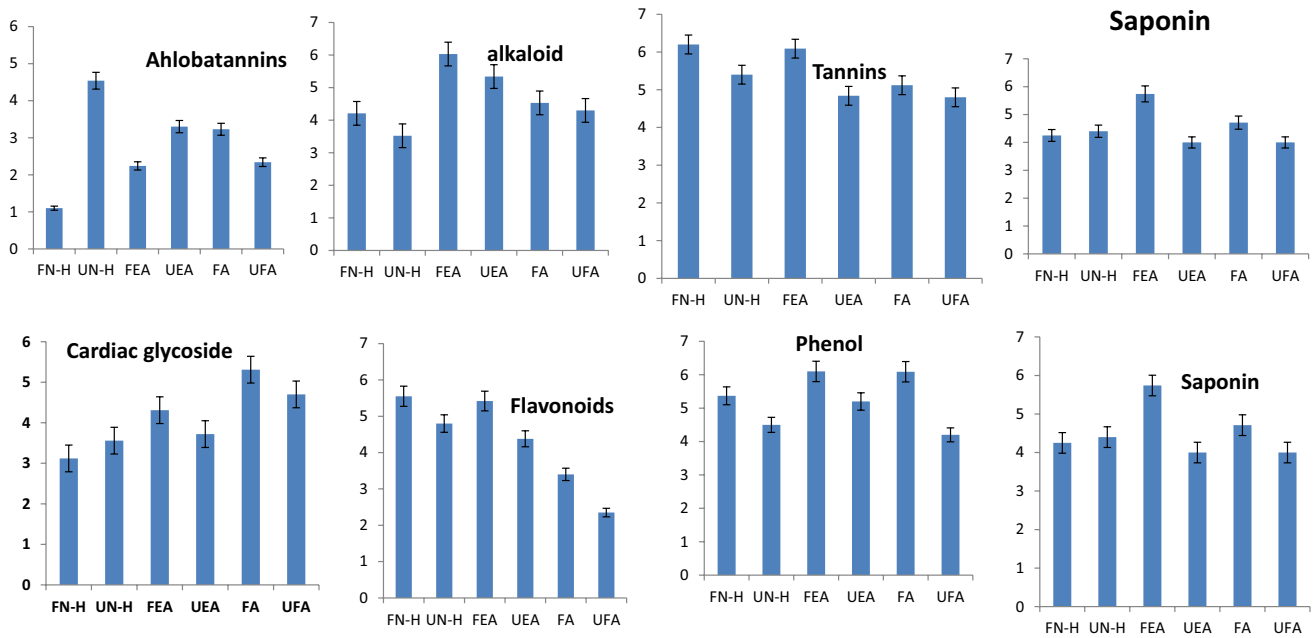
Figure 1 shows the quantitative phytochemical analysis of fermented and unfermented *P. biglobosa* seeds analyzed with N-hexane, Ethyl acetate, and acetone respectively. N-hexane extracted phytochemicals show tannins with the highest bar of 6.20 µg in 100 g of the fermented sample and 5.40 µg in the unfermented sample followed by flavonoids having 5.55 µg/100 g of the sample and 4.80 µg in the unfermented sample. Phenol shows a total of 5.20 µg/100 g in unfermented and increased drastically to 6.10 µg/100 g in the fermented sample, alkaloid, tannins, saponin, and flavonoid also shown to be in high concentrations as compared to the unfermented sample which is less than the fermented sample constituents. The chemical properties of the essential oil of *Parkia biglobosa* play a key role in its solubility in N-hexane.

Moreover, the availability of tannin, flavonoids, steroids, phenols, cardiac glycosides, Saponins, and alkaloids observed in the phytochemical screenings of the *Parkia biglobosa* (fermented and unfermented) is in agreement with the findings of Ogbole and Akinmoladun (2020) and this explains their therapeutic value. Steroids found in fermented *P. biglobosa* are accountable for the treatment of certain endocrine disorders, blood sugar regulation, salt imbalance, and microbial infections (Gautam et al. 2013). Furthermore, reducing sugar and anthraquinone are negative for fermented samples while steroids, phenol, and flavonoids were increased after fermentation (Fig. 1). The saponins and alkaloids found in *P. biglobosa* are effective in the treatment of Syphilis, Rheumatism, and certain skin diseases, including the treatment of abscesses and other swellings, ulcers, and septic wounds (Mallikharjuna et al. 2007). Saponins are in charge of tonic and stimulating Steroids that display analgesic properties (Soetan et al. 2014).

Cardiac glycosides are a type of naturally occurring drug that aids in the treatment of congestive heart failure. Glycosides are drugs that are found as secondary metabolites in medicinal plants and are used to treat congestive heart failure and cardiac arrhythmia (Gautam et al. 2013). Mostly in the Yoruba tribe of southwestern Nigeria, this class of phytoconstituents compound was discovered in *P. biglobosa* extracts, which aid in the therapies of heart infections as well as other ailments such as dental caries and cough.

## Quantitative mineral analysis of fermented and unfermented seeds of *Parkia biglobosa*

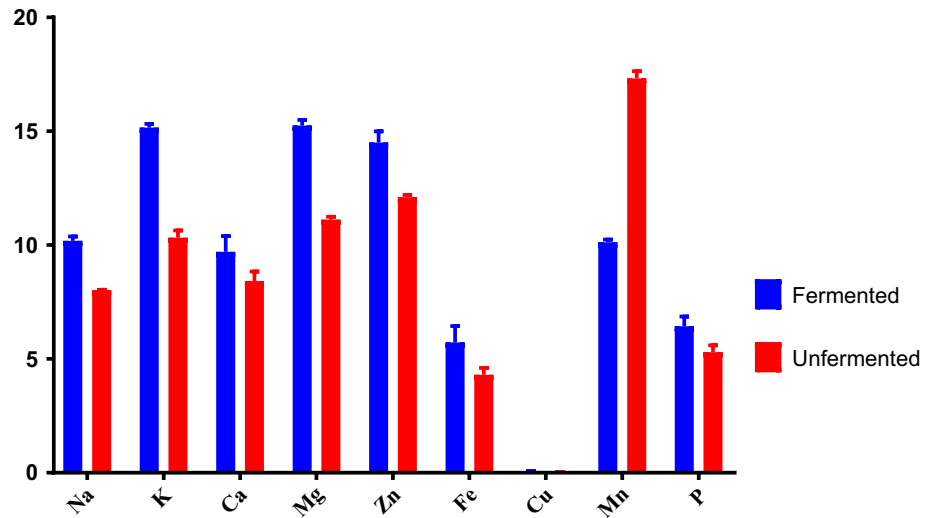
The quantitative analyses of minerals present in the fermented and unfermented samples of *Parkia biglobosa* are shown in Fig. 2. Magnesium was the most abundant



**Fig. 1** Quantitative phytochemical analysis of fermented and unfermented *Parkia biglobosa*. All the values are repeated in triplicate and reported as Mean  $\pm$  SD. *FN-H* fermented N-hexane, *UN-H* unfermented N-hexane, *FEA* fermented Ethyl acetate, *UEA* unfermented Ethyl acetate, *FA* fermented acetone, *UFA* unfermented acetone

mented N-hexane, *FEA* fermented Ethyl acetate, *UEA* unfermented Ethyl acetate, *FA* fermented acetone, *UFA* unfermented acetone

**Fig. 2** Quantitative mineral analysis of fermented and unfermented seeds of *Parkia biglobosa* ( $\mu\text{g}/100\text{ g}$ ). All the values are repeated in triplicate and reported as mean  $\pm$  SD



element in the fermented sample with 15.25  $\mu\text{g}/100\text{ g}$  of the sample, followed by potassium having 15.16  $\mu\text{g}/100\text{ g}$  and Zinc at 14.50  $\mu\text{g}/100\text{ g}$  while Sodium was 10.19  $\mu\text{g}/100\text{ g}$ , Manganese has 10.12  $\mu\text{g}/100\text{ g}$  followed by calcium with 9.70  $\mu\text{g}/100\text{ g}$ . Phosphorus and Iron were also abundant in 100 g of the sample with 6.43  $\mu\text{g}/100\text{ g}$  and 5.72  $\mu\text{g}/100\text{ g}$

respectively. Lead was absent in both the fermented and non-fermented samples. There is an increase in the number of the constituents' elements in the fermented than the unfermented sample.

Furthermore, the mineral composition showed the presence of beneficial elements in both the fermented and unfermented samples. The result from the study indicated that lead is absent in both fermented and unfermented samples (Fig. 2). Manganese is present in large quantities in both samples and is a microelement that is required for human nutrition which many enzymes are activated by it (Soetan et al. 2010). Copper is a micro-mineral that aids in iron absorption and red blood cell formation. It is also needed for enzyme production and biological electron transfer in the body (Soetan et al. 2010). Manganese aids blood clotting by collaborating with complex vitamins such as K and B vitamins. Manganese also aids in the management of stress. When an expectant mother does not get enough of this essential nutrient, birth defects may occur (Oluwaniyi and Bazambo 2016).

Subsequently, to maintain acid–base balance, the osmotic balance between cells and interstitial fluid, and nerve function, the body requires sodium. Its unavailability initiates mental apathy, muscle cramps, and a loss of appetite (Oluwaniyi and Bazambo 2016). The daily sodium allowance for healthy adults is no more than 2.3 g, even though the WHO recommended a reduction to < 2 g per day (Mente et al. 2021). Calcium is also required for normal cardiac muscle function, blood coagulation and clotting, and cell permeability regulation. Calcium deficiency symptoms include rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension, and uterine cramping (Oluwaniyi and Bazambo 2016).

**Table 2** Antioxidant analysis of fermented and non-fermented *Parkia biglobosa* seeds extracted using acetone

	Non-fermented	Fermented
Phenol mg/g	28.15 ± 1.7369 <sup>a</sup>	31.04 ± 0.0247 <sup>b</sup>
Flavonoid mg/g	4.25 ± 0.0343 <sup>a</sup>	6.68 ± 0.0269 <sup>b</sup>
FRAP mg/g	22.77 ± 0.0210 <sup>a</sup>	24.42 ± 0.0205 <sup>a</sup>
DPPH%	49.83 ± 0.1064 <sup>a</sup>	80.40 ± 0.0637 <sup>b</sup>
ABTS Mol/g	0.0028 ± 0.0001 <sup>a</sup>	0.0074 ± 0.0002 <sup>b</sup>
Fe <sup>2+</sup> chelating %	8.26 ± 0.6048 <sup>a</sup>	43.40 ± 0.5343 <sup>b</sup>
NO radical %	27.18 ± 1.6219 <sup>a</sup>	52.33 ± 1.5896 <sup>b</sup>
SO radical %	26.38 ± 0.9710 <sup>a</sup>	50.13 ± 0.4372 <sup>b</sup>

All the values are repeated in triplicate and reported as mean ± SD

The values with the same superscript letters across the row are statistically different at  $p < 0.05$

### Antioxidant analysis of fermented and non-fermented *Parkia biglobosa* seeds extracted using acetone

The mean deviation of the antioxidant analyses of both fermented and unfermented seeds of *P. biglobosa* showed that the fermented sample has the highest mean deviation (Table 2). The antioxidant, 2,2-diphenyl-1-picrylhydrazyl has the highest composition in 100 g of the sample with  $80.40 \pm 0.064^b$  compared to  $49.83 \pm 0.206^a$  of the unfermented sample. Phenol is measured as mg/g of the sample; it has  $31.04 \pm 0.025^b$  in fermented and  $28.15 \pm 1.737^a$  in the unfermented sample. The flavonoid measured in mg/g is  $6.69 \pm 0.027^b$  in the fermented sample and  $4.25 \pm 0.034^a$  in the unfermented sample. The Ferric reducing antioxidant power (FRAP) of the sample was reduced after fermentation. Free radical scavenging activity of *P. biglobosa* samples recorded in mol/g of the sample as  $0.0074 \pm 0.0002^b$  in fermented and  $0.0028 \pm 0.0001^a$  in unfermented while Fe<sup>+</sup> chelating % is measured as  $43.3961 \pm 0.5343^b$  in fermented and  $8.2592 \pm 0.6048^a$  in the unfermented sample.

However, significantly higher phenol content (31.04) in the fermented sample to 28.15 in the unfermented sample may indicate significant antioxidant potential in fermented more than unfermented sample. Phenols are powerful antioxidants and metal chelators, with hepatoprotective, anti-inflammatory, anti-allergic, antithrombotic, anti-carcinogenic, and antiviral properties (Komolafe et al. 2014).

### Proximate analysis of fermented and unfermented *Parkia biglobosa* seeds

The analysis of proximate indicated that fermentation positively affects the contents of nutrients in the *P. biglobosa*

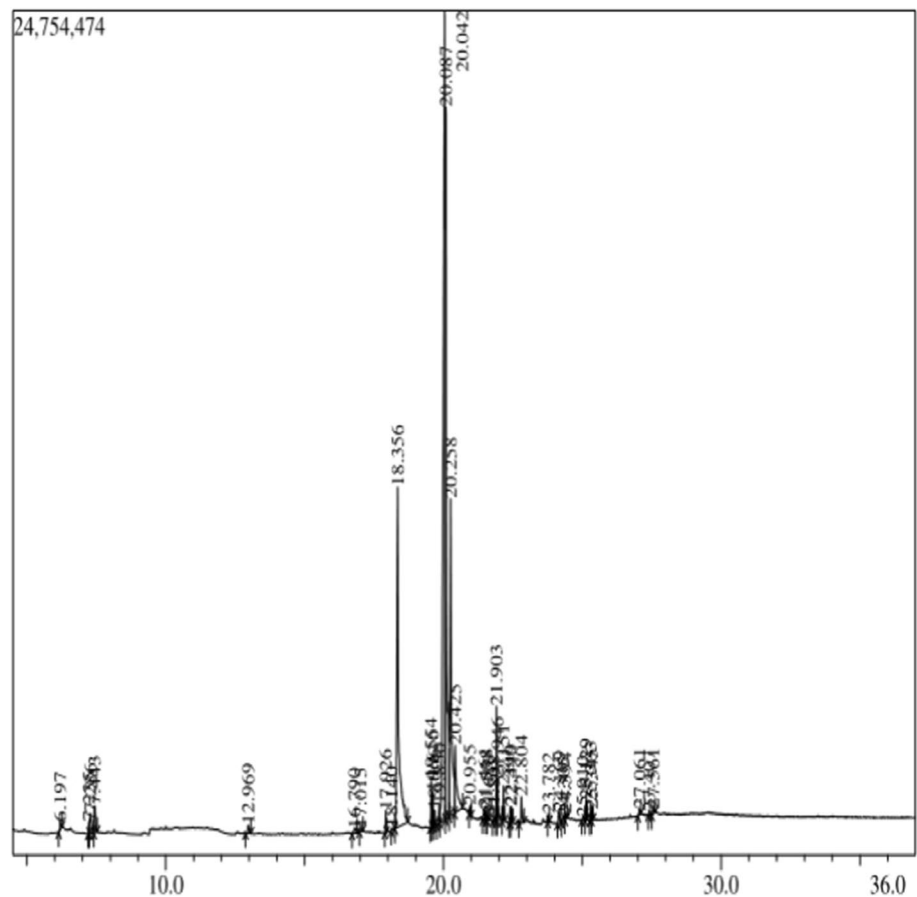
**Table 3** Proximate analysis of fermented and unfermented *Parkia biglobosa* seeds

Parameters	Dried unfermented	Dried fermented
Moisture content	8.14 ± 0.2703 <sup>a</sup>	15.00 ± 0.2250 <sup>b</sup>
Fat content	27.88 ± 0.3592 <sup>a</sup>	29.52 ± 0.3700 <sup>b</sup>
Fibre content	5.02 ± 0.6070 <sup>b</sup>	2.38 ± 0.3300 <sup>a</sup>
% Crude protein	38.54 ± 1.9541 <sup>a</sup>	44.95 ± 0.2800 <sup>a</sup>
Ash content	3.00 ± 0.1833 <sup>a</sup>	2.72 ± 0.1600 <sup>a</sup>
Carbohydrate	17.46 ± 1.8613 <sup>b</sup>	5.44 ± 0.3850 <sup>a</sup>

All the values are repeated in triplicate and reported as mean ± SD

At  $p < 0.05$ , values with the same superscript letters across the row are statistically different

**Fig. 3** Spectra of Gas Chromatography/Mass Spectrophotometry Analysis of essential oil in fermented *Parkia biglobosa* seeds



seeds sample (Table 3). Rises were detected in the content of Moisture ( $8.14 \pm 0.27$ – $15.00 \pm 0.23$  %), fat content ( $27.88 \pm 0.36$ – $29.52 \pm 0.37$  %) and the crude protein ( $38.54 \pm 1.95$ – $44.95 \pm 0.28$  %) after fermentation.

Moreover, the present study adequately explained the approximate composition of plant extracts. The extract contained different percentages of ash, moisture, crude protein, fat fiber, and carbohydrate. Because of the metabolic activities of microorganisms during the fermentation period, the moisture content of the fermented sample increases. The unfermented seeds' low moisture content (8.1367%) is comparable to that reported by Oluwaniyi and Bazambo (2016), ( $8.67 \pm 0.70\%$ ), and Omafuvbe et al. (2004), ( $8.6 \pm 0.6\%$ ). The fermented seed's high moisture content (51.54%) is comparable to that reported by Omafuvbe et al. (2004) ( $52.0 \pm 5.0\%$ ).

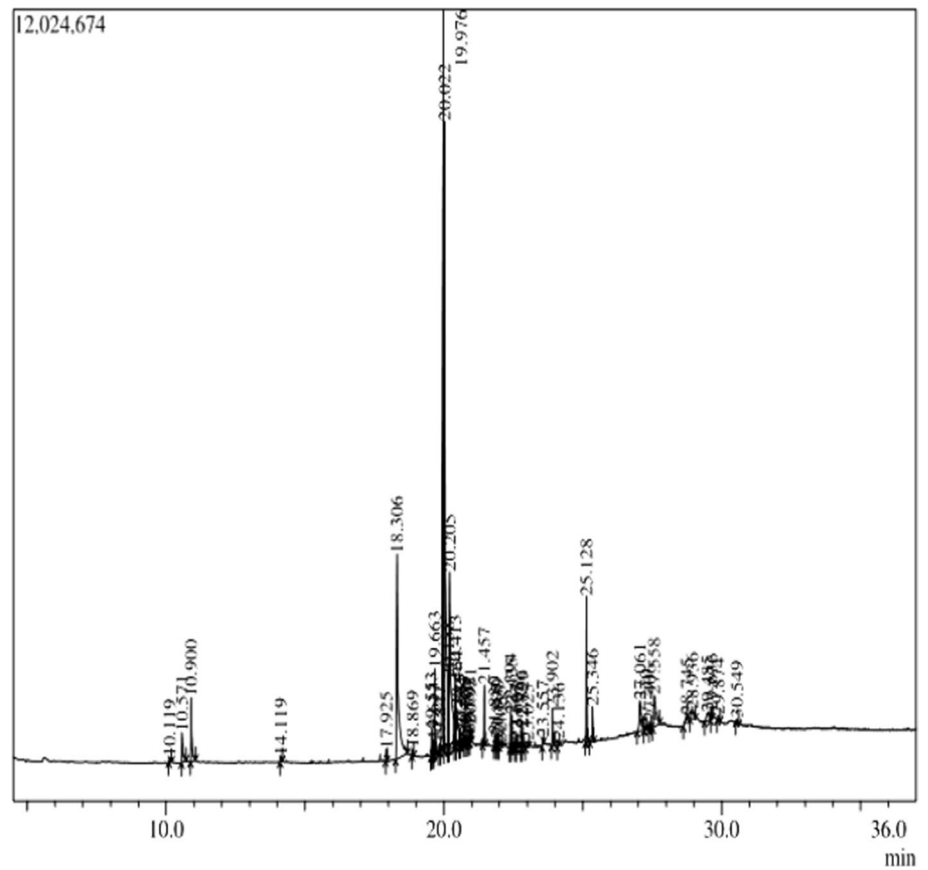
Figure 3 showed the GCMS spectra of the compounds identified in the essential oil extracts of fermented *P. biglobosa* with a main peak of about 37.83% area yield and match the library mass spectra to *9,12-Octadecadienoic*

*acid (methyl ester)*. Figure 3 also indicated *P. biglobosa* seed extracts contain essential oil with specific organic compounds such as Oleic acid and Palmitic acid which have been reported to be soluble in N-hexane and hence explained the solubility of Tannins and Flavanoids in N-hexane (Cao and Sofic 2006). Figure 4 revealed the GCMS spectra of the compounds identified in the essential oil extracts of unfermented *P. biglobosa* seeds, with the main peak of about 19.90% of the total area yield and matches the library mass spectra to *14-Pentadecanoic acid*. Ten compounds were detected in unfermented and fermented *P. biglobosa* seed essential oil to be of a peak, with *9,12-Octadecadienoic acid (methyl ester)*, *Pentadecanoic acid*, and *Stearic acid* being identified as marker compounds.

*Parkia biglobosa* essential oil extract showed the presence of large 7–8 peaks including *linoleic acid, methyl ester, pentadecanoic acid, arachidic acid, oleic acid chloride, Oleic anhydride, Vitamin E, Palmidrol, Urea, Palmitic acid, L-Ascorbic acid monostearate, and Glycerol 1,2 dipalmitate* (Table 4). Fatty acids with antibacterial



**Fig. 4** Spectra of Gas Chromatography/Mass Spectrophotometry Analysis of essential oil in non-fermented *Parkia biglobosa* seeds


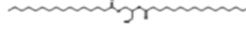
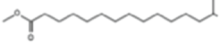
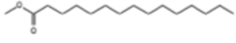
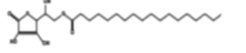

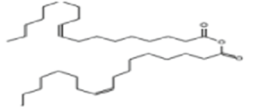
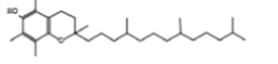
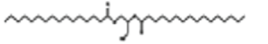



activities have been isolated from several plants using bioassay-guided fractionation, McGaw et al. (2002) described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum* which also proved why *P. biglobosa* has active inhibitory effects against similar organisms as reported. Linoleic and oleic acids inhibited the growth of Gram-positive *B. subtilis*, *Micrococcus kristinae*, and *S. aureus*, and linoleic acid also showed activity against *Bacillus cereus* and *Bacillus pumilis* (McGaw et al. 2002). *Parkia biglobosa* seed essential oil contains various non-polar organic compounds such as fatty acids, alkanes, terpenoids, and esters (Table 4). These non-polar compounds are highly soluble in non-polar solvents such as N-hexane which means they lack polar functional groups like hydroxyl (–OH) or carboxyl (–COOH) (Uche-Ikonne et al. 2019).

## Conclusion

The study concluded that *P. biglobosa* seed contains bioactive compounds that can be used to treat enteropathogenic bacterial infections and could also be used as an antioxidant source. The study concluded that the seeds extract of fermented and unfermented *P. biglobosa* were high in phytochemicals such as glycosides, flavonoids, tannins, cardiac steroids, saponins, and alkaloids. The study also concluded that fermentation enhanced the nutritional content of seeds of fermented *P. biglobosa*, which is needed to pave the way for the availability of nutrients and make the seeds eatable and its essential oil helped against bacterial activities as well.

**Table 4** Compounds identified in the essential oil of fermented and unfermented *Parkia biglobosa* seeds Gas Chromatography-Mass Spectrometry (GC-MS)

S/N	Compound	Formula R	Mol/W	R.Index	Peak	Structure
1.	Arachidic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	2366	390	
2.	Glycerol 1,2-dipalmitate	C <sub>35</sub> H <sub>68</sub> O <sub>5</sub>	568	4013	390	
3.	Palmitic acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	1878	487	
4.	Pentadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	1779	487	
5.	L-Ascorbic acid monostearate	C <sub>24</sub> H <sub>42</sub> O <sub>7</sub>	442	3346	390	
6.	14-Pentadecenoic acid	C <sub>15</sub> H <sub>28</sub> O <sub>2</sub>	240	1859	495	
7.	Oleic anhydride	C <sub>36</sub> H <sub>66</sub> O <sub>3</sub>	546	3919	476	
8.	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	3149	394	
9.	Dipalmitin	C <sub>35</sub> H <sub>68</sub> O <sub>5</sub>	568	4013	378	
10.	9,12-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	2093	435	

## Recommendation

It is recommended that *P. biglobosa* seed oil should be further studied phytochemically to elucidate the active principle in the fermented and unfermented seeds which can be used as a leading antibacterial agent in addition to its uses as food condiments.

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**Author contributions** VOI and TKA Conceptualization; DOO, OGO, and TKO writing original draft; VOI, ADD, and OGO writing, review, and editing. All authors read and approved the final manuscript.

**Data availability** All data generated or analyzed during this study are included in this published article.

## Declarations

**Conflict of interest** We authors declare that we have no competing financial interests.

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