Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 18 No. 2; pp. 1135-1146 (2020). ISSN (print): 1596-7409; eISSN (online):9876-5432

Antimicrobial activity of the crude extracts of *Parkia biglobosa* (Jacq) seeds on selected clinical isolates

Ajiboye AE and §Hammed BA

Microbiology Unit, Department of Biosciences and Biotechnology, Faculty of Pure and Applied Sciences. Kwara State University, Malete, Kwara State, Nigeria.

§Corresponding author: Hammed BA. Email: aishat.hammed@kwasu.edu.ng

Abstract

Parkia biglobosa (Jacq) is a wild leguminous plant found in North-Central zone of Nigeria with high calorific value, essential proteins, fatty acids, and vitamins. The study investigated the antimicrobial activity of crude extracts of fermented and unfermented *P. biglobosa* seeds on selected clinical microbial isolates namely, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. *P. biglobosa* seeds were obtained from Oja-Oba market in Ilorin, Kwara State, Nigeria. The samples were pre-treated and pulverized into powder. The extraction was achieved with acetone and water and qualitative phytochemical analysis was performed following standard procedures. The antimicrobial activity of the extracts against the isolates was determined by agar well diffusion method. Qualitative phytochemical screening of the crude extracts showed the presence of tannins, alkaloid, flavonoid, saponin and glycosides. *P. aeruginosa* was sensitive to the aqueous extract of fermented seeds having a zone of inhibition of 14.00±1.00mm while for unfermented seeds it was 10.00±2.00 mm at 100 mg/ml. The acetone extracts of both fermented and unfermented seeds revealed antibacterial activity against *P. aeruginosa* with zone of inhibition of 17.00±3.00 mm and 18.00±0.00 mm respectively. In conclusion, the crude extracts of the fermented and unfermented *P. biglobosa* seeds at a concentration of 75 and 100 mg/ml respectively have antimicrobial effect on the clinical isolates.

Keywords: *Parkia biglobosa*, Crude extracts, Phytochemicals, Antimicrobial, Clinical isolates

https://dx.doi.org/10.4314/br.v18i2.2 Open Access article distributed under the terms of the Creative Commons License [CC BY-NC-ND 4.0] http://creativecommons.org/licenses/by-nc-nd/4.0.
Journal Homepage: ***http://www.bioresearch.com.ng.***
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

INTRODUCTION

Parkia biglobosa also known as African locust beans is a dicotyledonous plant in the family Fabaceae – Mimosoideae. It is classified as a vascular plant (Thiombiano *et al*., 2012). African

locust bean is a leguminous crop commonly grown in the tropics. It is very prominent in West Africa and can be found in the Northern and South - Western regions of Nigeria (Tee *et al.,* 2009). Its height ranges between 7 and 20 metres (Fern, 2019). *P. biglobosa* is a leguminous plant commonly known for their readily available protein content, high calorific value, essential amino acids and fatty acids content, vitamin, and fiber. Fermented seeds are highly nutritional with several health benefits (Oloyede and Akintunde, 2019). However, the presence of anti-nutrients in the seeds has limited their use (Bhat and Karim, 2009).

P. biglobosa plants are rich sources of phytochemicals, wood, fuel and gum and the seeds have been investigated for their protein and amino acid contents as reported by Ajaiyeoba (2002). The tree has a thick dark brown bark and it is fire-resistant while the pods which house the seeds are usually 30-45 centimeters long on average and are dark brown when mature (Janick, 2008; Abioye *et al*., 2013).

The African locust bean is prepared locally by subjecting the seeds to natural fermentation of the boiled and de-hulled cotyledon. The different duration of fermentation of the seeds makes them edible by increasing their digestibility (Simonya, 2012). Fermentation involved in the preparation of the seeds promotes the desired nutritional value accentuates the organoleptic properties such as taste, flavour and texture (Akin-Osanaiye and Musa, 2017).

The seeds are widely used in Africa and usually the fermented forms are processed to condiments such as, iru, dawadawa, afitin, soumbala, netetu and sonru with high nutritive value (Zannou *et al*., 2018). The fermented seeds of *P. biglobosa* are used as condiments in preparing local soups in Nigeria and it is known as 'iru', 'origili' and 'dawadawa' among the Yorubas, Ibos and Hausas respectively (Dosumu *et al.,* 2012; Ojewunmi *et al*., 2016). In traditional medicine, seeds of *P. biglobosa* are used for the management and treatment of some infectious diseases; they are used as anti-malarial and anti-bacterial agents (Balogun *et al*., 2018). However, there is limited scientific investigation on the antibacterial activity, although there are reports that the main microorganisms involved in the fermentation process also help to inhibit harmful bacteria such as *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* (Ouoba *et al*., 2005).

Microorganisms such as *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* amongst others are becoming increasingly resistant to antimicrobial agents. Antibiotic resistance has been reported to occur when a drug loses its ability to inhibit bacterial growth effectively and this is increasingly becoming a global concern (O' Neil, 2016). Bacteria which have become 'resistant' continue to multiply in the presence of therapeutic levels of the antibiotics. Antibiotics which are usually effective against these microbes become less effective or the microbes become resistant as such, requiring a higher than the normal concentration of the same drug to have an effect. The emergence of antimicrobial resistance was observed shortly after the introduction of new antimicrobial compounds (Levy, 2017). Also, since antimicrobials are not fully degraded in human and animal body; antimicrobial compounds, their metabolites and transformation products are abundant in the environment (Segura *et al*., 2009; Michael *et al.*, 2013). Consequently, this leads to the selection of antimicrobial resistant bacteria or the acquisition of resistance genes by horizontal gene transfer (Martinez, 2009). Emergence of less effective antibiotics lead to the risks of many treatments failures (O'Neill, 2016), resulting in the need for an alternative therapy. Therefore, the aim of this study is to determine phytochemical constituents and the antimicrobial activity of crude extracts of fermented and unfermented *P. biglobosa* seeds on selected clinical isolates.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh samples of locust bean seeds (fermented and unfermented) were purchased from Oja-Oba market in Ilorin, Kwara State and placed in a clean plastic container during transportation to the laboratory. The fermented locust bean seeds were cleaned by hand picking of the dirt particles and other physical contaminants and was dried in an oven (DHG 9202) at 40 ^{0}C for 3 days. However, the unfermented seeds were first soaked in water and thoroughly rinsed, afterwards boiled in order to enhance the removal of hull from the cotyledons. The cotyledons obtained were then dried in an oven at 40 ^{0}C for 3 days. Both samples were then grounded with Flourish blender (FL1039) and kept in an airtight container prior to analysis.

Sample Extraction

Sterile warm water (40 ^{0}C) and acetone were used to obtain extracts from the fermented and unfermented locust bean seeds. Seed extracts were obtained by maceration; 125 grams of the sample was dissolved in 1000 ml of water and was left to stand for 24 hours. The extracts were then filtered using muslin cloth and the aqueous filtrates was evaporated to dryness using a water bath at a temperature of 40 ^{0}C for 3 hours while the filtrates obtained using acetone was dried in a fume cupboard. The crude warm water and acetone extracts of the seeds were then subjected to phytochemical and antimicrobial analysis (Oluwaniyi and Bazambo, 2014).

Test Microorganisms

The test microorganisms were clinical isolates obtained from University of Ilorin Teaching Hospital, Oke-Oyi, Ilorin, Kwara State. Bacteria obtained were *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* and fungus obtained was *Candida albicans.* The organisms were reconfirmed by sub-culturing and identification according to the standard methods of Cowan and Steel (l993).

Standardization of Inocula

McFarland standard was prepared by introducing 0.05 ml of 1% BaCl in 9.95 ml of 1% H_2SO_4, the solution was then shaken well (CLSI, 2009). The inocula were standardized by introducing 10 ml of nutrient broth into sterile test tubes which were then inoculated with the test organisms. Afterwards, the turbidity of the media containing the isolates were then adjusted by adding sterile water to match that of the McFarland standard which is equivalent to 1.5 X 10^8 CFU/ml.

Qualitative Phytochemical Screening of Crude Extracts

Alkaloids: Two grams of each extracts were heated on a boiling water bath with 2% Hydrochloric acid (50 ml), cooled, filtered, and treated with Mayer's reagent (5 drops). The samples were then observed for the presence of yellow precipitation or turbidity (Tyler, 1994).
Flavonoids: Two ml of 50% methanol was added to 4 ml of the crude extracts at a concentration of 100 mg/ml. After warming, magnesium fillings followed by few drops of concentrated hydrochloric acid were then added. A pink/red colour indicates the presence of flavonoid (Tyler, 1994).
Tannins: A portion of each extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution were added. A blue/green color indicates the presence of tannins (Ahmed *et al*., 2013).
Saponins: A small quantity (0.5g) of each extract was boiled with sterile distilled water (3 ml). The mixture was filtered, and 2.5 ml of the filtrate was added to 10 ml of the distilled water in a test tube, shake well for about 30 seconds and observed for frothing (Sofowora, 2008).
Glycosides: to 2 ml of each crude extract (at a concentration of 100mg/ml in methanolic solution), Fehlings reagent was added and boiled for two minutes. A brick red coloration indicates the presence of glycosides (Ahmed *et al*., 2013).

Antibacterial Activity of *P. biglobosa* Seed Extract against Selected Clinical Microbial isolates

The agar well diffusion method as described by Irobi *et al.* (1996) and Okeke *et al.* (2001) was used for the antibacterial test. A pure culture of each test organism was grown in nutrient broth for 18 hours at 37 ^{0}C. The broth culture was then standardized to match McFarland turbidity standard which was approximately 1.5 x $l0^8$ cfu/ml. One ml of inoculum was then used to seed 20 ml of cooled molten Mueller Hinton agar (MHA) medium in Petri dishes. A sterile cork borer (6mm in diameter) was used to dig wells equidistant from each other on the surface of the solidified MHA medium and 0.1ml of each extract of fermented and unfermented seeds at a concentration of 25, 50, 75 and 100 mg/ml was delivered into the wells. A control, 0.1ml of sterile distilled water and acetone were used. Antibiotics (streptomycin, ciprofloxacin and rocephin) and antifungal (sporanox) were used as positive controls. Duplicates of each plate were made and the plates were then incubated at 37 ^{0}C for 24 hours after which zones of inhibition detected were measured using a meter rule, the efficiency of the extracts is directly related to the level of clearance (Dairo and Adanlowo, 2010)

Antifungal Activity of *P. biglobosa* Seed Extract against Selected clinical microbial isolates

The methods of Perez *et al*. (1990); Murugesan *et al.* (2011) with a little modification was employed in the test. Sterile Mueller Hinton agar was aseptically poured into sterile Petri-dishes and allowed to solidify properly. The fungal broth culture was then standardized to match McFarland turbidity standard which was approximately 1.5 x $l0^8$ cfu/ml. Approximately 0.1 ml was spread on the surface of the agar using sterile spreader. Wells, 6 mm in diameter, were made with a sterilized cork borer and different concentrations (25, 50, 75 and 100mg/ml) of the extracts, sterile distilled water and acetone as controls were dispensed, incubation was performed at 27 ^{0}C for 48 hours. Antifungal activity was determined by measuring the zones of inhibition produced by the extracts and sporanox was used as the positive control. Tests were conducted in duplicate to ascertain the results obtained.

Antibiotic Sensitivity Test

The *in vitro* antibiotic susceptibility testing of bacterial isolates was performed using the standardized disc agar diffusion methods (Kaiser, 2012; CLSI, 2013). The test was carried out using 0.1ml of standardized inoculum of each isolate which was spread evenly across the plates containing Muller Hinton agar. A sterile forcep was used to place the antibacterial disc on the media containing the culture, which was then incubated at 37 ^{0}C for 24 hours. The zones of inhibition around the disc were measured using a meter rule. The discs used were streptomycin (30mg), ciprofloxacin (10mg) and rocephin (25mg). The disc contained-septrin (30mg), chloramphenicol (30mg), pefloxacin (10mg), gentamycin (10mg), ampiclox (30mg), zinnacef (20mg), amoxicillin (30mg), rocephin (25mg), ciprofloxacin (10mg), streptomycin (30mg) and erythromycin (10mg).

Antifungal Sensitivity Test

Antifungal susceptibility test was performed on the fungal isolate using sporanox® (100 mg), which was adjusted to a concentration of 25 mg/ml. Sterile cork borer was used to bore two holes equidistance from each other on sterile solidified MHA plate containing the fungal isolate. Two drops of the adjusted concentration were then introduced into each well and the zone of inhibition obtained afterwards was measured.

Determination of Minimum Inhibitory Concentration (MIC) of the clinical isolates

The MIC of each extracts was determined as follows; the plant extracts used were aqueous and acetone extracts of the fermented and unfermented seed, 1 ml of each of the different concentrations- 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml was introduced into 1 ml of sterile nutrient broth tubes containing the test organisms, the tubes were then incubated at 37 ^{0}C for bacterial isolates and 28 ^{0}C for fungal isolate for 24 and 48 hours respectively. Tubes without plant extracts were used as positive control (Akintobi *et al*., 2016a).

Determination of Minimum Bactericidal and Fungicidal Concentration of the clinical isolates

Inoculum from the tubes showing no turbidity was introduced on a sterile nutrient agar and Sabourand dextrose agar for bacteria and fungi, respectively. The least concentration that produced no growth on the medium was taken as the minimum bactericidal concentration for the bacterial isolates and minimum fungicidal concentration for the fungal isolate (Ibekwe and Ezeji, 2011).

Data Analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using IBM SPSS (V21). The data were analyzed using one-way analysis of variance followed by LSD post-hoc test and independent samples T-test for comparison of means. $P < 0.05$ was considered significant.

RESULTS

Phytochemical Analysis of Extracts

Phytochemical analysis of the crude extracts shows the presence of tannins, alkaloid, flavonoid, saponin and glycosides in the acetone and aqueous extract of the fermented *P. biglobosa* seeds; alkaloid and tannin were present in the acetone extract of unfermented seeds and alkaloid, tannin, saponin and glycosides in aqueous extract of unfermented seeds. The result obtained from the qualitative phytochemical analysis of the extracts of both fermented and unfermented seeds of *P. biglobosa* is shown in Table 1.

Table 1: Qualitative phytochemical analysis of the crude extracts of *P. biglobosa* seeds

Phytochemicals	AFS	AUFS	AQFS	AQUFS
Alkaloid	+	+	+	+
Flavonoid	+	-	+	-
Tannin	+	+	+	+
Saponin	+	-	+	+
Glycosides	+	-	+	+

Key: + , present; - , absent; AFS, acetone extract of fermented seeds; AUFS, acetone extract of unfermented seeds; AQFS, aqueous extract of fermented seeds; AQUFS, aqueous extract of unfermented seeds

Antimicrobial Activity of Aqueous Extract of *P. biglobosa* Seeds against Selected Clinical Isolates

As shown in Table 2, the mean zone of inhibition obtained for the aqueous extract of fermented *P. biglobosa* seeds against *C. albicans* was highest at 100 mg/ml with a value of 11.50±1.50 mm and lowest at 25 mg/ml with a value of 0.00 mm; for *P. aeruginosa*, the highest zone observed at 100mg/ml was 14.00±1.00 mm and least zone at 25 mg/ml was 6.00±1.00 mm. Similar result was observed with *E. coli*; highest zone of 13.00±1.00 mm at 100 mg/ml and 6.50 ± 050 mm for *S. aureus*, 11.00±1.00 mm at 100 mg/ml and 0.00 mm at 25 mg/ml.

The mean zone of inhibition obtained for the aqueous extract of unfermented *P. biglobosa* seeds against *C. albicans* was highest at 100 mg/ml with a value of 11.00±1.00 mm Similar result was observed with *E. coli* and *S. aureus* with the highest zone of 11.00±1.00 mm at 100mg/ml as shown in Table 3.

Table 2: Mean zone of inhibition (mm) for aqueous extract of fermented *P. biglobosa* seeds against selected clinical isolates

	Concentration (mg/ml)/Mean Zone of Inhibition (mm)			
Clinical Isolate	25	50	75	100
C. albicans	0.00^{abc}	7.00 ± 1.00^{ad}	9.00 ± 1.00^{b}	11.50 ± 1.50^{cd}
P. aeruginosa	6.00 ± 1.00^{acd}	8.00 ± 2.00^{b}	12.50 ± 0.50^{c}	14.00 ± 1.00^{bd}
E. coli	6.50 ± 0.50^{abc}	10.00 ± 0.00^{ad}	11.00 ± 1.00^{b}	13.00 ± 1.00^{cd}
S. aureus	0.00^{acd}	7.00 ± 3.00^{b}	10.00 ± 2.00^{c}	11.00 ± 1.00^{d}

Values are expressed as mean ± SEM of duplicate readings of the zone of inhibition obtained for each isolate. Values with the same superscript in a row are statistically significant at $P < 0.05$

Table 3: Mean zone of inhibition (mm) for aqueous extract of unfermented *P. biglobosa* seeds against selected clinical microbial isolates

	Concentration (mg/ml)/Mean Zone of Inhibition (mm)			
Clinical Isolates	25	50	75	100
C. albicans	0.00^{acd}	0.00^{be}	6.50 ± 1.50^{bcf}	11.00 ± 1.00^{def}
P. aeruginosa	0.00^{abc}	6.50 ± 1.50^{a}	8.00 ± 2.00^{b}	10.00 ± 2.00^{c}
E. coli	0.00^{abc}	7.00 ± 1.00^{ade}	10.00 ± 0^{bd}	11.00 ± 1.00^{ce}
S. aureus	0.00^{abc}	5.50 ± 0.50^{ade}	9.00 ± 1.00^{bd}	11.00 ± 1.00^{ce}

Values are expressed as mean ± SEM of duplicate readings of the zone of inhibition obtained for each isolate. Values with the same superscript in a row are statistically significant at $P < 0.05$

Antimicrobial Activity of Acetone Extract of *P. biglobosa* Seeds against Selected Clinical Microbial Isolates.

The result obtained for the fermented crude acetone extract of *P. biglobosa* seeds revealed that the mean zone of inhibition obtained with *C. albicans* was highest at 100 mg/ml with a value of 13.50±1.50 mm. For *P. aeruginosa*, the highest zone of inhibition was 17.00±3.00 at 100 mg/ml and for *E. coli*, the highest zone of inhibition was 15.00±1.00 mm at 100 mg/ml as shown in Table 4.

For the unfermented crude acetone extract, the mean zone of inhibition obtained with *C. albicans* was highest at 100 mg/ml with a value of 13.00±1.00 mm for *P. aeruginosa*, the highest zone of inhibition was 18.00±0.00 mm at 100 mg/ml, for *S. aureus,* the highest zone of inhibition was 10.50±0.50 mm at 100 mg/ml as shown in Table 5.

Table 4: Mean zone of inhibition (mm) for acetone extract of fermented *P. biglobosa* seeds against selected clinical microbial isolates

	Concentration (mg/ml)/Mean Zone of Inhibition (mm)			
Clinical Isolates	25	50	75	100
C. albicans	0.00^{abc}	8.00 ± 2.00^{a}	10.00 ± 2.00^{b}	13.50 ± 1.50^{c}
P. aeruginosa	5.00 ± 1.00^{a}	11.00±1.00	14.00±4.00	17.00 ± 3.00^{a}
E. coli	5.00 ± 1.00^{a}	8.00 ± 0.00^{b}	11.00 ± 1.00^{a}	15.00 ± 1.00^{ab}
S. aureus	6.00±2.00	8.00±2.00	12.00±2.00	14.50±3.50

Values are expressed as mean ± SEM of duplicate readings of the zone of inhibition obtained for each isolate. Values with the same superscript in a row are statistically significant at $P < 0.05$

Table 5: Mean zone of inhibition (mm) for acetone extract of unfermented *P. biglobosa* seeds against selected clinical microbial isolates

	Concentration (mg/ml)/Mean Zone of Inhibition(mm)			
Clinical Isolates	25	50	75	100
C. albicans	0.00^{abc}	7.00 ± 1.00^{ad}	11.00 ± 1.00^{bd}	13.00 ± 1.00^{c}
P. aeruginosa	5.50 ± 0.50^{a}	10.50 ± 0.50^{a}	15.00 ± 1.00^{a}	18.00 ± 0.00^{a}
E. coli	0.00^{acd}	5.50 ± 0.50^{be}	10.00 ± 2.00^{c}	12.00 ± 2.00^{de}
S. aureus	0.00^{abc}	5.50 ± 0.50^{ad}	9.50 ± 1.50^{bd}	10.50 ± 0.50^{c}

Values are expressed as mean ± SEM of duplicate readings of the zone of inhibition obtained for each isolate. Values with the same superscript in a row are statistically significant at $P < 0.05$

Minimum Inhibitory Concentration of Aqueous Extract of Fermented and Unfermented *P. biglobosa* Seeds against Selected Clinical Isolates

The Minimum Inhibitory Concentration (MIC) obtained for the fermented aqueous extract for *S. aureus* and *C. albicans* was 75 mg/ml while the MIC for *P. aeruginosa* and *E. coli* was 50 mg/ml. The same result was obtained for the MIC of the unfermented aqueous extract as shown in Table 6. The Minimum Inhibitory Concentration (MIC) obtained for the fermented acetone extract with *S. aureus* was 100 mg/ml and 75 mg/ml for *C. albicans*, *E. coli* and *P. aeruginosa*. However, the MIC obtained for the unfermented acetone extract of *P. biglobosa* seeds with *S. aureus* was 75mg/ml and 50mg/ml for *C. albicans*, *E. coli* and *P. aeruginosa* as shown in Table 6.

Table 6: Minimum inhibitory concentration of aqueous and acetone extract of fermented and unfermented *P. biglobosa* seeds against selected clinical microbial isolates

	P. biglobosa Seeds/MIC (mg/ml)			
	Aqueous extract		Acetone extract	
Clinical Isolates	Fermented	Unfermented	Fermented	Unfermented
S. aureus	75	75	100	75
P. aeruginosa	50	50	75	50
E. coli	50	50	75	50
C. albicans	75	75	75	50

Minimum Bactericidal Concentration and Minimum Fungicidal Concentration of Crude Extracts of *P. biglobosa* Seeds against Selected Clinical Microbial Isolates

The minimum bactericidal concentration (MBC) of the aqueous and acetone extracts of fermented and unfermented *P. biglobosa* seeds against *S. aureus* was 100 mg/ml, 75 mg/ml and 50 mg/ml respectively while for *E. coli*, the MBC obtained using aqueous extract of fermented and unfermented seeds was 50mg/ml and 75mg/ml respectively while that of the acetone extract of fermented and unfermented seeds was 50mg/ml for both extracts. The Minimum Fungicidal Concentration (MFC) obtained with

C. albicans using aqueous extract of fermented seeds, aqueous extract of unfermented seed and acetone extract of unfermented seeds was 75mg/ml while for the acetone extract of fermented seeds, the MFC was 50mg/ml, as shown in Table 7.

Table 7: Minimum bactericidal and fungicidal concentration of aqueous and acetone extract of fermented and unfermented *P. biglobosa* seeds against selected clinical microbial isolates

Clinical Isolates	AQFS	AQUFS	AFS	AUFS
S. aureus	100mg/ml	75mg/ml	-	50mg/ml
P. aeruginosa	100mg/ml	-	-	50mg/ml
E. coli	50mg/ml	75mg/ml	50mg/ml	50mg/ml
C. albicans	75mg/ml	75mg/ml	50mg/ml	75mg/ml

Key: AQFS, aqueous extract of fermented seeds; AQUFS, aqueous extract of unfermented seeds; AFS, acetone extract of fermented seeds; AUFS, acetone extract of unfermented seeds.

Antibiotic and Antifungal Sensitivity Testing against Selected Clinical Isolates

The result obtained from antibiotic sensitivity test performed using commercial antibiotic disc is shown in the Table 8; Streptomycin (S), inhibited all the bacterial isolate with a mean zone of inhibition of 13.00±1.00 mm for *P. aeruginosa*, 16.00±2.00 mm for *E. coli* and 14.00 ±1.00 mm for *S. aureus*; Ciprofloxacin inhibited *P. aeruginosa* with a mean zone of inhibition of 14.00±1.00 mm, 17.50 ±2.50 mm for *E. coli* and 15.00 ± 0.00 mm for *S. aureus*. However, antifungal sensitivity test using Sporanox (25 mg/ml) gave a mean zone of inhibition of 20.00±0.00 mm against *C. albicans*.

Table 8: Mean Zone of Inhibition obtained using Commercial Antibiotics/Antifungal against Selected Clinical Microbial Isolates

	Mean Zone of Inhibition (mm) ± SD			
Clinical isolates	S	CPX	R	SP
C. albicans	-	-	-	20.00±0.00
P. aeruginosa	13.00±1.00	14.00±1.00	20.00±0.00	-
E. coli	16.00±2.00	17.50±2.50	11.50±1.50	-
S. aureus	14.00±1.00	15.00±0	0.00	-

Key: S, streptomycin; CPX, ciprofloxacin; R, rocephin; SP, sporanox; SD, standard deviation

DISCUSSION

Qualitative phytochemical analysis of both the fermented and unfermented aqueous and acetone extracts of *P. biglobosa* seeds revealed the presence of various phytochemicals, with the fermented aqueous and acetone extracts of the seeds revealing the presence of all the phytochemicals studied than the unfermented extracts. The difference in phytochemical constituents may be attributed to fermentation which might have made the phytochemicals readily available in the fermented extracts. Phytochemicals are secondary constituents of plants which are responsible for biological actions and some have also been reported to possess anti-oxidative potentials (Oluwaniyi and Bazambo, 2014). The absence of flavonoids in the unfermented aqueous and acetone extract of *P. biglobosa* seeds is in line with the study of Oluwaniyi and Bazambo (2014). Phytochemicals are particularly implicated in antimicrobial potentials of various medicinal plants as supported by different researchers.

Antimicrobial analyses of the fermented aqueous extract of *P. biglobosa* seeds revealed that increasing concentration tends to increase

the mean zone of inhibition; a similar result was observed with *E. coli*. Also, increasing the concentration tends to increase the mean zone of inhibition obtained with *S. aureus*, these results are in agreement with the study conducted by Akintobi *et al*. (2016b) which opined that the higher the concentration of plant extracts, the higher the antimicrobial activities. However only the least concentration as well as the highest concentration was statistically significant, the same was observed against *P. aeruginosa*. This may be due to the fact that the aqueous extract contains phytochemicals such as tannins, alkaloids, saponins and glycosides which may be responsible for this effect. This result agrees with the work of Tijani *et al* (2009) who reported that the aqueous stem bark extract of the same plant had inhibitory effect on the test organisms. The aqueous extract of fermented seeds had the most significant inhibitory effect on *E. coli* and *S. aureus*, this could be as a result of the ability of the aqueous solvent to extract the phytochemicals which is responsible for the antimicrobial as well as antioxidant activity of the extracts. This is in line with the work of Ibekwe and Ezeji (2011) who reported that the aqueous root and leaf extract of the same plant showed inhibitory effect on *S. aureus* and *E. coli*.

Antimicrobial activity of the acetone extract of the fermented *P. biglobosa* seed showed no significant difference in the mean zone of inhibition obtained with *S. aureus* at all concentrations; for *P. aeruginosa*, a significant difference was only observed with the least concentration and the highest concentration. However, increasing concentration tend to produce a slight increase in the mean zone of inhibition obtained with *C. albicans* and *E. coli*. This tally with the work of Dosumu *et al.* (2012) who reported that the methanolic extract of *P. biglobosa* seeds had inhibitory effect on the test organisms. The unfermented seeds also showed inhibitory effect on the test organisms but with significant effect on *P. aeruginosa, C. albicans* and *S. aureus*. However, for *E. coli*, there was only a slight significant difference in the mean zone of inhibition with increasing concentration. This shows that the extract contains some active phytochemicals which when purified could serve as a potential drug to combat these highly resistant microorganisms. This agrees with the work of Ajaiyeoba (2002), who reported that the aqueous extract of the leaves of the same plant had inhibitory effect on the test organisms. Analysis of the bactericidal and fungicidal activity of the extracts revealed that the acetone extract of fermented *P*. *biglobosa* seeds was bactericidal on all the organisms except *S. aureus* and *P. aeruginosa*. Also, the aqueous extract of the unfermented seeds was not bactericidal on *P. aeruginosa*. This may be due to the highly resistant nature of these organisms or probably because the concentration was not high enough to kill them. The aqueous extract of fermented seeds and the acetone extract of unfermented *P*. *biglobosa* seeds were bactericidal on all the test organisms. *Candida albicans* was susceptible to the commercial antifungal while the antibiotics were effective against both gram positive and Gram-negative bacteria as observed also by Akintobi *et al.* (2016b).

CONCLUSION

The crude extracts of both the fermented and unfermented seeds of *P. biglobosa* showed antimicrobial activity on the selected clinical isolates. The aqueous extract of fermented seeds had more inhibitory effect on *E. coli* and *C. albicans* while the acetone extract had pronounced inhibitory effect on *P. aeruginosa* and *S. aureus*. The result also shows that water was a better extracting solvent than acetone as it had a significant inhibitory effect on all the organisms than acetone extracts. The study therefore confirms the folklore use of the seeds of *P. biglobosa* for the treatment of infections that could arise from these microorganisms.

REFERENCES

Abioye, E., Akinpelu, D., Aiyegoro, O., Adegboye, M. and Okoh, A. (2013). Preliminary phytochemical screening and antibacterial properties of crude stem bark extracts and fractions of *Parkia biglobosa* (Jacq). *Molecule* (18): 8485-8499.

Ademola, I., Baiyewu, R., Adekunle, E., Omidiran, M. and Adebayo, F. (2011). An assessment into physical & proximate analysis of processed locust bean (*Parkia biglobosa*) preserved with common salt. *Pakistan Journal of Nutrition*. 10(5): 405-408.

Ahmed, S., Hanifi, S. and Iftkhar, T. (2013). Phytochemical profiling with antioxidant and antimicrobial screening of *Amaranthus viridis*, L. leaf and seed

extracts. *Open Journal of Medicinal Microbiology*. 3: 164-171.

Ajaiyeoba, E. (2002). Phytochemical and antibacterial properties of *Parkia biglobosa and Parkia bicolor* leaf extracts.*Afr. J. Biomed. Res.* 5: 125 – 129.

Akin-Osanaiye, B. and Musa, A. (2012). Assessment of biochemical and antimicrobial activity of bacteria associated with fermented locust beans in Manyare community of Kogi State, Nigeria. *Direct Research Journal of Agriculture and Food Science*. 5(8): 291-296.

Akintobi, O., Bamkefa, B., Adejuwon, A. and Fagbbola, T. (2016a). Phytochemical analysis and antimicrobial evaluation of the extracts of the root bark and rachis of *Parkia biglobosa* (Jacq). Benth on some human pathogens. *E3 Journal of Scientific Research*. 4(1); 001-010.

Akintobi, O.A., Bamkefa, B.A., Adejuwon, A.O. and Oduola, S.O. (2016b). Antimicrobial evaluation of extracts of leaf and root wood of *Parkia biglobosa* (Jacq.) Benth. against some human pathogenic bacteria. *Life Science Journal* 13(11): 55 - 63

Amyes, S. (2007). Enterococci and Streptococci. *Int. J. Antimicrob Agents*. 29 Suppl 3: S43–52. doi:10.1016/S0924-8579(07)72177-5. PMID 17659211.

Bhat, R. and Karim, A. (2009). Exploring the nutritional potential of wild and unidentifed legumes. *Comprehensive Review Food Science and Food Safety*. 8(4): 305-331.

Brownlee, H., McEuen, A., Hedger, J. and Scott, I. (1990). Anti-fungal effects of cocoa tannin on the witch broom pathogen *Crinipellis perniciosa*. *Physiol. Molec. Plant Pathol.* 36:39.

Clinical and Laboratory Standards Institute (2009). Performance Standards for Antimicrobial Disk Susceptibility Tests; *Approved Standard* - Tenth Edition. 556-557.

CLSI (2013). Update on the 2012-2013 CLSI. Standards for Antimicrobial Susceptibility Testing: Edition-Susan Sharp.http://www.swacm.org/annulmeeting/2012/stlouisworkshops. WS4GP. CLSIupdate2012.pdf.

Dairo, F.A.S. and Adanlowo, I.G. (2010). Nutritional quality of *Crassocephalum crepidioides* and *Senecio biafrae*. *Pakistan Journal Nutrition* 6:35-39.

Dosumu, O. Oluwniyi, Awolola, G. and Oyedeji, O. (2012). Nutritional composition and antimicrobial properties of three nigerain condiments. *Nigeria Institute of Food Science and Technology Journal*. 30(1): 43-52.

Evans, W. and Evans (2009). Pharmacognosy. 16th Ed. London: Saunders Elsevier. 385.

FDA. (2018). FDA safety changes: ketoconazole linked with liver injury. Medscape. WebMD LLC. Available at https://www.medscape.org/viewarticle/809250.

Fern, K. (2019). Useful Tropical Plants-*Parkia biglobosa*. Available at http://tropical.theferns.info/viewtropical.php?id=*Parkia+biglobosa*.

Hidron, A., Edwards, J., Patel, J., Horan, T., Sievert, D., Pollock, D. and Fridkin, S. National Healthcare Safety Network Team; Participating National Healthcare Safety Network Facilities. (2008). NHSN annual update: antimicrobial-resistant pathogens associated with healthcare associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007". Infect Control Hosp Epidemiol. 29(11): 996–1011. doi:10.1086/591861. PMID 18947320.

Ibekwe, V. and Ezeji, E. (2011). Antimicrobial activity of leaf and root extracts of *Parkia biglobosa* (African Locust Bean). *The African Journal of Plant Science and Biotechnology*. 5(1); 70-72.

Iyamu, M., Ekozien, M. and Omoigberale, M. (2014). Phytochemical screening and antibacterial activity of the stem bark of African locust bean plant (*Parkia filicoidea* Welw). *Global Journal of Biology, Agriculture and Healtth Sciences.* 3(2): 36-43.

Janick, J. (2008). *Parkia biglobosa* African Locust Bean. The encyclopedia of fruit & nuts (pp. 395-400). Wallingford, U.K.: CABI North American Office.

Kaiser, G. (2012). Kirby-Baver Test (Online Manual, Lab 21). Syllabus-Department of Biology-Western, Kentucky University. http://student.ccbcmd.edu/ngKaiser/index.html.

Kumamoto, C. (2002). *Candida* biofilms. *Current Opinion in Microbiology*. 5(6): 608–11. doi:10.1016/s1369-5274(02)00371-5. PMID 12457706.

Levy, S. and Marshall, B. (2004). Antibacterial resistance worldwide: causes, challenges, and responses. *Nat Med*. 10:122–129. doi: 10.1038/nm1145.

Levy, S. (1998). The challenge of antibiotic resistance. *Sci. Am.* 278: 46–53.

Levy, S. (2017). Antibiotic Resistance: An ecological imbalance, in ciba foundation symposium - antibiotic resistance: origins, evolution, selection and spread. Chadwick DJ, Goode J. John Wiley & Sons, Ltd. (ed): Chichester, UK, 2007. 1(1):14. doi: 10.1002/9780470515358.ch1.

lwu, M. (1993). *Handbook of African Medicinal Plants*. CRC Press, Boca Raton, Fl. pp. 106.

Maranan, M., Moreira, B., Boyle-Vavra, S. and Daum, R. (1997). Antimicrobial resistance in staphylococci: Epidemiology, molecular mechanisms, and clinical relevance. *Infect Dis Clin North Am*. 11:813–849. Doi:10.1016/S0891-5520(05)70392-5.

Martinez, J. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution.* 157: 2893–2902.

Masih, U., Shrimali, R. and Naqvi, S. (2012). Antibacterial activity of acetone and ethanol extracts of *Cinnamon* (*Cinnamomum zeylanicum*) and Ajowan (*Trachyspermum ammi*) on four food spoilage bacteria**.** *International Research Journal of Biological Sciences.* 1(4):7-11.

Michael, I., Rizzo, L., McArdell, C., Manaia, C., Merlin, C., Schwartz, T., Dagot, C. and Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Res.* 47; 957–995.

Molander, A., Reit, C., Dahlén, G. and Kvist, T. (1998). Microbiological status of root-filled teeth with apical periodontitis. *International Endodontic Journal*. 31(1): 1–7. doi:10.1046/j.1365-2591.1998. t01-1- 00111.x . PMID 9823122.

Murugesan, S., Annamalai Pannerselvam, A. and Tangavelou, A.C. (2011). Phytochemical screening and antimicrobial activity of the leaves of *Memecylon umbellatum* burm. F. *Journal of Applied Pharmaceutical Science* 01 (01): 42-45

Musa, H. (1991). Ginger and Locust bean tree: History, growth, use and potentials. paper presented at Tuk Ham Symposium. Kurmin Musa. 29-30.

Ojewumi, M., Omoleye, A. and Ajayi, A. (2016). The effect of different starter cultures on the protein content in fermented African Locust bean (*Parkia biglobosa*) seeds. *International Journal of Engineering Research and Technology (IJERT)*. 5 (4).

Okeke, M., Iroegbu, C. Eze, E., Okoli, A. and Esimone, C. (2001). Evaluation of *Extracts of the Root of Landolphia owerience* for antibacterial activity. *Journal of Ethnopharmacology*. 78: 119-127.

Oloyede, G.K. and Akintunde, T.I.(2019). Fatty acids profile, physicochemical properties and antioxidant activity of unfermented and fermented *Parkia biglobosa* (African Locust beans) seed oil *J. Chem Soc. Nigeria*, 44(2): 178 -186

Oluwaniyi, O. and Bazambo, I. (2014). Anti-nutrition and phytochemical evaluation of raw and fermented African locust bean (*Parkia biglobosa*) seeds. *Global Journal of Pure and Applied Science*. 20: 105-109.

Ouoba, L., Diawara, B., Annan, N., Poll, L. and Jakobsen, M. (2005). Volatile compounds of Soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. *J Appl Microbiol*. 99:1413–1421.

Perez, C. Pauli, M. and Bazerque, P. (1990). An antibiotic assay by agar well diffusion method. *Acta Biol. Med. Exp*. 15: 113-115.

Rocas, I., Siqueira, J. and Santos, K. (2004). Association of *Enterococcus faecalis* with different forms of periradicular diseases. *Journal of Endodontics*. **30** (5): 315–320. doi:10.1097/00004770-200405000-00004

Salit Bako, S., Onche, E., Mbonu, I., Olotu, P. and Lajide, L. (2014). *Parkia biglobosa* plant parts; phytochemical, antimicrobial, toxicity and antioxidant characteristics. *Journal of Natural Sciences Research*. 4(2).

Santhi, R., Lakshmi, G., Priyadharshini, A. and Anandaraj, L. (2011). Phytochemical screening of *Nerium oleander* leaves and *mormodicachrantia* leaves. *Inter. Res. J. Pharm*. 2: 131-135.

Segura, P., Francois, M., Gagnon, C., and Sauve, S. (2009). Review of the occurrence of anti-infectives in contaminated wastewaters and natural and drinking waters. *Environ Health Perspect.* 117: 675–684.

Simonya, K. (2012). African locust bean Seed processing; Prospects, status and challenges. ISBN 978-978-923-050-5.

Sofowora A. 2008. Phytochemical screening. Medicinal plants and traditional medicine in Africa, 3rd ed. Spectrum books limited, Ibadan, Nigeria, p. 199-204.

Tee, T., Ogwuche, J. and Ikyaagba, E. (2009). The role of Locust bean and Ironwood trees in human nutrition and income in Nigeria. *Pakistan Journal of Nutrition*. 8(8): 1172-1177.

Thiombiano, D., Lamien, N., Dibong, D., Boussim, I. and Belem, B. (2012). The role of woody species in managing food shortage in Burkina Faso. *Sécheresse*. 23(2): 86-93.

Tijani, A., Okhale, S., Salawu, T., Onigbanjo, H., Obianodo1, L., Akingbasote, J., Salawu, O., Okogun, J., Kunle, F. and Emeje, M. (2009). Antidiarrhoeal and antibacterial properties of crude aqueous stem bark extract and fractions of *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don. *African Journal of Pharmacy and Pharmacology*.3(7); 347-353.Available online http://www.academicjournals.org/ajpp.

Tyler, V. (1994). Phytomedicines in Western Europe; their potential on herbal medicine in the United States. *Herbalgram*. 30: 24-30.

Zannou, O., Chabi, I.B. and Koca, I. (2018). Some traditional fermented foods from African locust beans (*Parkia biglobosa*): production and volatile compounds. *Global Scientific Journal.* 6(9): 673-701