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Research Paper

Phytochemical Analysis and Antimicrobial Activities of the Extracts of the fruit husk of *Parkia biglobosa* (African locust bean)

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***ABSTRACT**: Phytochemical screening and elemental analysis were carried out on water, acetone and methanol extracts of fruit husk of Parkia biglobosa. The antimicrobial activity of the extracts was tested against some gram positive and gram negative bacterial and fungal organisms. The Minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) and the minimum inhibitory concentration ratio (MBC/MIC and MFC/MIC) was determined. Preliminary phytochemical investigation revealed the presence of alkaloids, cardiac glucosides, flavonoids, saponin, phlobotannin, terpenoids, reducing sugar, volatile oil, tannins and phenols. Elemental analysis revealed the presence of Lead (Pb), Manganese (Mn), Cupper (Cu), Potassium (K), Sodium (Na), Iron (Fe), Magnesium (Mg) and Calcium (Ca). Iron (Fe) was present at higher concentration of 15 mg/kg followed by Sodium (Na) 10 mg/kg, copper (Cu) 0.4 mg/kg, and zinc (Zn) 0.3 mg/kg in trace quantities. Antimicrobial studies revealed that acetone extract has considerable activities against the bacteria Staphylococcus aureus, Streptococcus pyogenes and Salmonella typhi at the dose of 400 and 500 µg/kg with a recorded inhibition zones range between 7.7 to 11.7 mm and inhibitory ratio (MBC/MIC) of 1.33. Water extract revealed activity against Streptococcus pyogenes and Escherichia coli at the dose of 300, 400 and 500 mg/kg with inhibitory zones ranging between 7.0 to 14.7 mm and the inhibitory ratio (MBC/MIC) of 1.25. Anti-fungal activity of acetone extract against Candida albicans was revealed at the doses 200, 300, 400 and 500 mg/kg with inhibition zones range between 8.33 to 16.7 mm and inhibition ratio (MFC/MIC) of 1.5. The activity of water extract against Candida albicans was revealed at the doses of 100, 200, 300, 400 and 500 mg/kg with the inhibition zones range between 7.18 to 18.7 mm and inhibition index (MFC/MIC) of 2.0. Methanol extract appears not to have any activity against the microorganisms tested in this experiment.*

***KEY WORDS**: Parkia biglobosa, fruit husk, phytochemical, antimicrobial activity, inhibitory zones and inhibitory ratio.*

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I. INTRODUCTION

The use of herbs or plant products by man as medicine has been from time immemorial. Herbal preparation represents one of the important traditional therapies in the world and it is still the mainstay. The World Health Organization (WHO) reports that more than 80 % of the world's population rely on traditional medicine for their primary health care needs especially in developing countries [19]. An estimated 25 % of the modern medicines are made from plants, first used as traditional medicine in the treatment of diseases [13]. In rural areas, there are additional cultural factors that encourage the use of herbal preparations; people believe that where a particular disease is endemic, there shall always be plants to cure such ailment within the that environment. Other factors are, absence of primary health care centers, lack of competent staff, poor diagnostic facilities and inadequate supplies of medicines [9].

Due to the development of adverse effects and microbial resistance to the chemically synthetic drugs, there is a global shift to ethno-pharmacognosy. Many health beneficial activities of plants such as anticancer,

antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported as a result of studies conducted on medicinal plants [34]. Recently, medicinal plants have become the focus of intense study in terms of conservation and their traditional medicinal uses. The studies on plants have either supported the claims by the traditional healers or contradicts their folkloric believe [23, 1].

Diseases caused by bacteria, fungi, viruses and helminths are highly prevalent in societies of low socio-economic status. They are common in Africa, the middle and Far East, Central and South America and other tropical regions of the world, but less common in developed countries of the West [22, 16].

Antimicrobial agents, especially antibacterial and antifungal agents are used globally in livestock production systems to protect the health of livestock and to improve their productivity. Antimicrobial resistance (AMR) is currently one of the major threats in medicine. The emergence and rapid spread of multi and pan drug resistant organisms to antibiotics (such as vancomycin, methicillin, extended spectrum β-lactam, carbapenem and colistin-resistant organisms) has put the world in a dilemma. Available antimicrobials have been misused and are almost ineffective with some of them associated with dangerous side effects in some individuals. Development of new effective and safe antimicrobials is one of the ways by which AMR burden can be reduced. The rate at which microorganisms develop AMR mechanisms out-spaces the rate at which new antimicrobials are being developed [21].

Parkia biglobosa is a tree belonging to the genus Parkia in the family Fabaceae. The tree has a wide distribution across the Sudan and Guinea savanna ecological zones. It is found in 19 African countries [12]. The tree is popularly known as African locust bean tree it is also known as "Dorawa" in Hausa, "Irugba" in Yoruba, "Origili" in Ibo [2]. *Parkia biglobosa* is a large tree of up to 7-20 m in height with dense spreading crown. It is a perennial deciduous tree; it can reach 30 m in exceptional cases. It leaves alternates, dark green in color and bipinnate up to 30 cm long. The Pinnae are up to 17 pairs with 13-60 leaflets. The leaflets are oblong to linear 0.8-3.0 cm x 0.2-0.8 cm, the flowers are red or orange color, they cluster in heads up to 7 cm in diameter, the borne hanging peduncle are up to 10-35 cm long. Pods are linear and can be 12-30 cm x 15-25 cm glabrous brown. The seeds are globosely-ovoid 5-15 mm, smooth and glossy, and dark in color. There are about 2800-6700 seeds per kilogram, the seeds are hard coated and can remain viable for up to 8 years. The seed when fermented can serve as cooking ingredient by women in most African countries and is refer to as dawa-dawa in Hausa land [62]. The plant has a very slow growth and begins fruiting in the 8th year [32, 38, 14]. At 15-20 years old it produces 25-100kg of pods per tree.

The fruit husk of *Parkia biglobosa* is widely used in North Eastern Nigeria to treat variety of microbial infections but there is limited documented information about the use of this plant for these purposes. This study was designed to provide information which will either invalidate or support the folkloric usage of *Parkia biglobosa* fruit husk as an antibacterial or antifungal agent, it will also serve as a guide to the potential users of the plant.

The objectives of this study therefore are to determine the Phytochemical and elemental constituents of the fruit husk of *Parkia biglobosa* extracts (water, methanol and acetone extracts) and to determine the activity of the extract against some bacterial and some fungal organisms.

II. MATERIALS AND METHOD

Plant collection, identification and extracts preparations:

A branch of *Parkia biglobosa* tree containing the leaves, stem and fruits was collected from Chibok town in Borno state, Nigeria and was submitted for confirmation and authentication by a plant taxonomist, a voucher specimen was deposited in the department of veterinary pharmacology and toxicology, Federal University of Agriculture Makurdi, Benue state, Nigeria where this research was conducted. The fruit husk was removed from the fruits and air dried at room temperature and thereafter pulverized by grinding with pestle and mortar. Using the cool macerated method as described by Umeh *et al*., (2005) [41], the three different extracts were processed. The solvents used for the extractions were water, methanol and acetone. Two hundred grams (200 g) of the pulverized plant material was weighed and mixed with one liter (1 L) of the solvents listed above in separate conical flasks respectively. The mixture was allowed to stay for four days (4 days) and then filtered using Whatman No. 1 filter paper. The extracts were recovered separately by evaporating the solvents using water bath at 45 ^{O}c and the yield was calculated respectively. The extracts were then collected, labelled and stored at 4 ^{0}C until used.

Determination of extraction yield:

The extraction yield in % was calculated as follows;

$$\text{Extraction yield} = \frac{\text{Weight of extract after evaporating solvent and freeze drying}}{\text{dry weight of the sample}} \text{ x } 100$$

Determination of elemental constituents of fruit husk of *Parkia biglobosa*:

The elemental composition was determined by a standard method as described by Sunderman, 1973 [36], Kolthoff and Elving, 1976 [18]. A beam atomic absorption spectrophotometer (Philip/pye Unicom Ltd, England) was used to determine the elements at their appropriate wave length, temperature and lamp current [42]. The elemental concentrations were determined by a standard calibration curve.

Qualitative Phytochemical analysis:

The extracts of water, methanol and acetone of the fruit husk of *Parkia biglobosa* were freshly prepared and divided into different test tubes, the various constituents were analyzed according to the methods described by Harbone (1984), Evans (2002) and Sofowora (2008) [11, 10, 35]. The plant metabolites that were tested for includes, alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, steroids, phlobotannins, terpenoids, reducing sugar, volatile lipid, tannins and phenols.

Test microorganism and growth media:

The microorganisms used for this research are; bacteria strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and fungal strains of *Aspergillus niger* and *Candida albicans*, they are chosen based on their clinical and pharmacological importance [25]. The bacterial and fungal stock cultures used for this experiment were obtained from the Department of Veterinary Medicine, University of Maiduguri. The cultures for bacteria and fungi were incubated for 24 hours at 37 °C Mueller Hilton agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India) respectively and stored at 4 °C.

Antimicrobial activity:

The agar disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) for bacteria testing (CLSI, Performance Standards for Antimicrobial Disc Susceptibility Tests, 2012) was used to determine the antibacterial activity of the plant extracts. The agar plates were inoculated with a standardized inoculum of the test microorganisms on separate plates. Filter paper discs (6 mm in diameter) containing the plant extracts at different concentrations of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml were placed on the agar surface with the help of a sterile forceps, chloramphenicol 5 µg/ml disc was used as positive standard and 10 % DMSO as a negative standard. The Petri dishes were incubated at 37 ^{0}C for 24 h in an incubator. Generally, the antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism. The diameters of the inhibition growth zones were measured with ruler at three different dimensions and recorded accordingly in millimeter (mm).

Antifungal activity:

Agar well diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) for yeasts testing (CLSI, Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, 2004) was used to evaluate the antifungal activity of the plants extracts. Similar to the procedure used in disc-diffusion method for antibacterial test, the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. A hole with a diameter of 6 to 8 mm was punched aseptically using a sterile cork borer, and some volumes of the extract solution at different concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml were introduced into the well accordingly, Ketoconazole at 200 mg/ml was use as standard. The agar plates were then incubated at 20-25 ^{0}C for 7 days. The plant extract diffuses in the agar medium to inhibit the growth of the microbial strain tested. The inhibition growth zones were then measure with a ruler in millimeter (mm) at three different dimensions and recorded accordingly.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

The tube dilution method [27,4] was used to determine the MIC of the extracts in both the bacterial and fungal organisms respectively. The prepared working concentrations (500, 400, 300, 200 and 100 µg/ml) for the bacteria and the same working concentration of the extracts in mg/kg for the fungal organisms were placed in different test tube containing nutrient agar. The tubes were then inoculated with 0.1ml of standard inoculum and incubated for 20-24 h at 37°C to observe turbidity (growth). The least concentration which showed no visible sign of growth and showed no turbidity of the medium was considered as the MIC.

The MBC and MFC were determined by sub-culturing the contents of the test tubes onto sterile nutrient agar plate respectively using a wire loop and the inoculated plates was incubated at 37 °C for 24 h. The MBC and MFC values were recorded as the least concentration that totally killed the test organisms, indicated by the complete absence of growth of the organism [29]. The ratio of MIC/MBC and MIC/MFC was then calculated and recorded accordingly.

Statistical analysis:
The zones of inhibition of the microorganisms were recorded in triplicate. The mean and standard error of mean (mean±SEM) of the zones of inhibition were calculated and recorded appropriately. The SPSS (IBM) version 23 statistical package was used for the statistical analysis.

III. RESULTS

Extraction:
The extraction of *Parkia biglobosa* fruit husk using water, methanol and acetone gave the following yields respectively; Water extract 16 %, Methanol extract 10 % and Acetone extract 15 %.

Mineral Composition of the fruit husk of *Parkia biglobosa*
The mineral elements and their composition present in the fruit husk of *Parkia biglobosa* is represented in Table 1.0., the result showed; Iron (Fe) 15.0 mg/kg had the highest concentration, followed by sodium (Na) 10.0 mg/kg and copper (Cu) 0.4 mg/kg and zinc (Zn) 0.3 mg/kg in trace quantities. The concentration of potassium (1.5 mg/kg) and sodium (Na) was found to be above the WHO (1996) standard. Some toxic heavy metals such as lead and cadmium were also present in trace amounts.

Table 1.0. Mineral Composition in The Fruit Husk of *P. biglobosa*

Mineral element	Composition (mg/kg)	WHO (1996) Standard (mg/L or ppm)
Lead (Pb)	0.4	1 - 2
Manganese (Mn)	2.5	10 - 20
Cupper (Cu)	0.4	1 - 3
Potassium (K)	1.5	0.1 - 1.0
Sodium (Na)	10.0	4 - 5
Iron (Fe)	15.0	0.5 - 50
Magnesium (Mg)	1.0	10 - 20
Calcium (Ca)	2.5	360 - 800
Cadmium (Cd)	0.3	10 - 20
Zinc (Zn)	0.3	15 - 20

Qualitative Phytochemical Analysis:
The phytochemicals present in the three different extracts (acetone, methanol and water) of the fruit husk of *Parkia biglobosa* and their degree of availability is presented in Table 2.0.
The result showed that acetone and methanol extracts have the high presence of alkaloids, flavonoids and phenols. The water extract contained high presence of flavonoids and terpenoids. Tannins were revealed to be high in the acetone and methanol extracts but lower in water extract while saponins only occurred in the water extract.

Table 2.0. Phytochemical Constituents of Crude Extracts of Acetone, Methanol and Water Extracts of *P. biglobosa* Fruit Husk

Phytochemical Constituents	Extracts		
	Acetone	Methanol	Water
Alkaloids	++	++	+
Anthraquinones	–	–	–
Cardiac glycosides	+	+	+
Flavonoids	++	++	++
Saponins	–	–	+
Steroids	–	–	-
Phlobotannins	–	+	–
Terpenoids	+	–	++
Reducing sugar	+	+	+
Volatile oil	+	+	–
Tannin	++	++	+
Phenol	++	++	+

Key: (-) = Negative, (+) = Positive, (++) = Highly positive

***Invitro* antimicrobial activity of the crude extracts:**

The antibacterial activity shown by acetone, methanol and water extracts of fruit husk of *Parkia biglobosa* is represented in Table 3.0. The acetone extract inhibited the growth of S*treptococcus pyogenes*, *Salmonella typhi* and *Staphylococcus aureus*, but has no effect on the growth of *Escherichia coli.* The zones of inhibition were greater with *Streptococcus pyogenes* and *Staphylococcus aureus* than with *Salmonella typhi*. The aqueous extract inhibited *Streptococcus pyogenes* and *Escherichia coli*. The zone of inhibition by the aqueous extract was greater with *Streptococcus pyogenes* than with *Escherichia coli*. The inhibition of the organisms by the acetone and aqueous extracts appeared to be concentration dependent. The highest concentration (500 µg/ml) produced the greatest inhibitory effect while the lowest concentration gave lesser or no inhibitory effect. The methanol extract did not inhibit any bacterial growth. Chloramphenicol which is a standard antibacterial gave the highest zones of inhibition in all the organisms tested, thus has the greatest activity than those produced by the extracts of fruit husk of *P. biglobosa*.

Table 3.0. The mean* Antibacterial Activity of Extracts of Acetone, Methanol and Water of Fruit Husk of *P. biglobosa*

Crude extracts	**Conc. (µg/ml)**	**Antibacterial activity (mm) on various organism**			
		S. pyogenes	***E. coli***	***S. typhi.***	***S. aureus***
Acetone.	500	11.0±0.0	R	10.0±0.0	11.7±0.33
	400	7.7±0.33	R	8.6±0.0	8.7±0.33
	300	R	R	R	R
	200	R	R	R	R
	100	R	R	R	R
Methanol	500	R	R	R	R
	400	R	R	R	R
	300	R	R	R	R
	200	R	R	R	R
	100	R	R	R	R
Water	500	14.7±0.33	11.7±0.33	R	R
	400	12.0±0.0	9.0±0.0	R	R
	300	8.7±0.3.	37.0±0.0	R	R
	200	R	R	R	R
	100	R	R	R	R
10% DMSO		R	R	R	R
Chloramphenicol (30 µg/ml)		35.0±0	18.0±0	28.0±0	20.0±0

R- Resistant, *mean±SEM based on 3 observation

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of acetone, methanol and water of fruit husk of *P. biglobosa*

The result of the MIC of acetone extract of the fruit husk of *P. biglobosa* against *Streptococcus pyogenes*, *Salmonella typhi* and *Staphylococcus aureus* bacteria is 300 µg/ml while MIC of water extract against *Streptococcus pyogenes* and *E. coli* is 400 µg/ml (Table 4.0). There was no growth of the bacteria following sub-culture of the contents of the tubes above the MIC. The antibacterial activities of the acetone and water extracts on the microbial organisms were considered bactericidal because MBC/MIC ratios obtained was $\leq$ 4. If the MBC/MIC $\geq$ 4 the activity will be considered bacteriostatic [60, 61].

Table 4.0. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Acetone, Methanol and Water Extracts of Fruit Husk of *P. biglobosa* on Some Bacteria

Extract	Test Organism	MIC (µg/ml)	MBC(µg/ml)	MBC/MIC
Acetone Extract	*S. aureus*	300	400	1.33
	S. pyogenes	300	400	1.33
	E. coli	-	-	-
	S. typhi	300	400	1.33
Methanol Extract	*S. aureus*	-	-	-
	S. pyogenes	-	-	-
	E. coli	-	-	-
	S. typhi	-	-	-
Water Extract	*S. aureus*	-	-	-
	S. pyogenes	400	500	1.25
	E. coli	400	500	1.25
	S. typhi	-	-	-

Invitro* antifungal activity of the acetone, methanol and water extracts of fruit husk of *P. biglobosa

The *invitro* antifungal activities of acetone, methanol and water extracts of the fruit husk of *P. biglobosa* is presented in the table 5.0 The acetone and aqueous extracts of *Parkia biglobosa* inhibited the growth of *Candida albicans*, but had no effect on the growth of *Aspergillus niger*. The zones of inhibition produced by the water extract at various concentrations were greater than those of acetone extracts. The inhibition produced by both the acetone and aqueous extracts of *P. biglobosa* on *Candida albicans* appear to be concentration dependent. The highest concentration (500 mg/ml) gave the greatest inhibitory effect while the lowest concentration gave the least inhibitory effect. Ketoconazole which is a standard antifungal drug that was used in this experiment inhibited both *Candida albicans* and *Aspergillus niger*. The zone of inhibition produced by Ketoconazole against the fungal organisms was greater than those produced by the acetone and water extracts. Methanol extract had no effect on the fungal organisms used in this experiment.

Table 5.0. *Invitro* Mean* Antifungal Activity of Acetone, Methanol, and Water Extracts of *P. biglobosa* on Some Fungal Organisms

Crude extracts	Conc. (mg/ml)	Zones of inhibition (mm)	
		Candida albicans	***Aspergillus niger***
Acetone.	500	16.7±0.33	R
	400	13.3±0.33	R
	300	10.7±0.33	R
	200	8.33±0.33	R
	100	R	R
Methanol	500	R	R
	400	R	R
	300	R	R
	200	R	R
	100	R	R
Water	500	18.7±0.33	R
	400	14.7+0.33	R
	300	11.7±0.33	R
	200	8.7±0.33	R
	100	7 .0±0.0	R
10% DMSO		R	R
Ketoconazole (50 mg/ml)		22.33±0.33	30.0±0.0

R- Resistance, *Mean+SEM based on 3 observations

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts of fruit husk of *P. biglobosa.*

The MIC and MFC of the extracts of *P. biglobosa* are shown in Table 6.0. The MIC of the acetone and water extracts against *Candida albicans* are 100 mg/ml and 50 mg/ml respectively while the MFC for the acetone extract is 150 mg/ml and for water extract is 100 mg/ml. MFC/MIC ratio of the acetone extract against *Candida albicans* was 1.5 while that of water extract was 2.0, indicating that the extracts are fungicidal because the MIC/MFC ratio is $\leq$ 4. Methanol extract has no effect against the fungal organisms used in this study.

Table 6.0. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Extracts of Fruit Husk of *P. biglobosa* on Some Fungal Organisms

Extract	Test organism	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC
Acetone extract	*Candida albicans*	100	150	1.5
	Aspergillus niger	-	-	-
Water extract	*Candida albicans*	50	100	2.0
	Aspergillus niger	-	-	-
Methanol extract	*Candida albicans*	-	-	-
	Aspergillus niger	-	-	-

IV. DISCUSSION

The resultant of extracts of fruit husk of *Parkia biglobosa* showed differences in extraction yields, the differences in polarity of solvents could be responsible for the variations observed. The solvents affected the extraction yield and the content of bioactive substances in each of the extracts, this might as well be responsible for the different effects shown by the activities of the extracts [26]. The maximum extraction yield was obtained from the water extract (16 %), followed by acetone extract (15 %) and methanol extract (10 %). Based on the result obtained by Ishnava *et. al*., (2015) [43], the percentage yield obtained in this experiment may be considered high. Other factors responsible for the difference in extraction yields may include, the phytochemicals present in the plants, the extraction temperature, the extraction time and solvent to solute ratio of plant [44].

The elements present in the plant at different concentrations were; Lead (Pb), Manganese (Mn), Cupper (Cu), Potassium (K), Sodium (Na), Iron (Fe), Magnesium (Mg), Calcium (Ca), Cadmium (Cd) and Zinc (Zn). The presence of essential elements is known to influence various body functions due to their direct actions in physiological and toxic concentrations [45]. Elements such as Ca, Fe, and Mg are known to play essential roles in human and animal health. Plants containing Fe and Cu such as *Moringa oleifera* are used for correction of anemic conditions, while those containing Ca are responsible for their ability to rebuild weak bones [45]. Zn is known to be present in every part of the body and has a wide range of functions, it is involved in healing of wounds and is an important component of many enzymes. It is particularly important for healthy skin, healthy immune system and resistance to infection [46]. The concentration of the toxic inorganic metals such as Cadmium (Cd) and Lead (Pb) present in this plant were far lesser than the maximal permissible addition (MPA) of the heavy metals and metalloids which are 0.76 mg/kg for Cadmium and 55 mg/kg for Lead [47]. This result may be an indication of low degree of pollution in the area where this plant was sampled. Chibok town is not an industrial area, therefore industrial activities leading to waste disposal of toxic elements were absent. The presence of all these elements in the soil is within the standard safety margin reported by WHO (1996). The inorganic elements (Mg., Zn., Ca., Fe., Na., and K) present in the plant have important role in health and diseases of both man and animals as have been highlighted by Abdulrahman, 2004 [59]. The high concentration of Fe (15 mg/kg) in this plant could positioned it to be considered as a good source of dietary supplement for Fe. Fe is an essential component in oxygen binding to hemoglobin and also act as catalyst for many enzymes like cytochrome oxidase [58]. In conclusion the presence these elements in this plant may influence the levels of the mineral constituent in the body system of man and animal when consumed [24].

The varied phytochemicals present in the fruit husk of *Parkia biglobosa* includes; alkaloids, cardiac glycosides, flavonoids, saponin, terpenoids, reducing sugar, volatile oil, phenolics and tannins. Alkaloids were found present in all the three extracts; the clinical importance of alkaloids include muscle relaxation and hence promote sleep [15]. It also has an antibacterial, antimalaria, antihypertensive and anticancer activities [8]. Aporphine alkaloids was observed to have anthelmintic activity [48]. Cardiac glycoside was also present in all the three extracts, clinically they increase the inotropic effect and the rate of contraction of the heart. Cardiac glycosides have been utilized in the treatment of congestive heart failure, additionally they possess therapeutic properties of laxatives, diuretics and antiseptic [33]. Flavonoids were present in all the extracts, they are reported to exert multiple bioactive properties such as possessing an antioxidant, antiviral, antibacterial, anticancer, anti-inflammatory and anti-cytotoxic properties [40]. Flavonoid is a potential reducing agent that protect the body cells from oxidative damage. Saponins were present only in the water extract of *Parkia biglobosa*, it has the property

of an expectorant and is very effective in the treatment of inflammation of the upper respiratory tract [49]. Saponin also possess antimicrobial property and can be used in the treatment of microbial infections [6]. Terpenoid was present only in water and acetone extracts but was higher in water extract, it has medicinal properties of anticancer, antiulcer, antimalaria and diuretic [20]. Reducing sugar was present in all the extracts, some reducing sugars can soothe the gastrointestinal tract and help in preventing diarrhea and gastroenteritis [7]. Volatile oil was present in acetone and methanol extracts of the plant, it has an antioxidant, antifungal, antimicrobial, antianxiety and pain reliving activities [28]. Tannin possess anticarcinogenic and antimutagenic activities, this may be related to the fact that they also have antioxidative properties which may be important in protecting cellular oxidation [50]. Tannins also have antimicrobial activity, the growth of yeasts, fungi, bacteria and viruses have been reported to be inhibited by tannins. Phenols have antioxidants, antibacterial, anthelmintic, and antineoplastic activities [51]. Their antioxidant capacities are related to the hydroxyl groups and phenolic rings present in the phenolic compound structure. They are also known to be powerful chain breaking antioxidants, which may contribute directly to oxidative action [52]. The result of the phytochemical study in this research supports that of the previous works conducted by Ajaiyeoba, (2003) [2] on the leave, stem bark and the root of *P. biglobosa* respectively.

The *invitro* antibacterial activity of the extracts showed that the acetone extract has activity against gram positive (*S. aureus*, *S. pyogenes)* and a gram-negative (*S. typhi)* organism. Methanol extract showed no activity against the bacterial organisms tested while, water extract also had activity against *Strep. pyogenes* and *E. coli* organism. MBC/MIC ratio of the acetone extract is 1.33 and that of water extract is 1.25 indicating that both extracts are bactericidal in activity. The MBC/MIC ratio of ≤ 4 is considered bactericidal while MBC/MIC ratio of > 4 is considered as bacteriostatic [53]. The antibacterial activity exhibited by this plant extracts may be related to the presence of phytochemicals such as, tannin, glucosides, saponins, and flavonoids which were confirmed to be present in this plant. The antimicrobial activities associated with these phytochemicals was reported by Akintobi, (2013) [3]. The findings of this research are also in agreement with the work of Adewale *et. al*., (2012) [54], who revealed that the stem bark of *Parkia biglobosa* has an antibacterial effect. Muhammed *et. al*., (2021) [55], in a review paper also revealed the antibacterial properties of *Parkia biglobosa*. The effectiveness of the acetone and water extracts on Staphylococcus organisms in this study is remarkable finding because multiple antibiotic resistance strains of these organisms exist in clinical setting worldwide [56].

The antifungal activity of these extracts was shown in acetone and water extracts against *Candida albicans*. The MFC/MIC ratio of acetone and water extracts against the activities of *Candida albicans* are 1.5 and 2.0 respectively, indicating that acetone and water extracts are either fungicidal. Methanol extract did not show any activity against fungal organisms tested in this experiment. This result supports the study conducted by Bello *et. al*., (2019) [57], that showed that the leaves and stem bark of *Parkia biglobosa* were effective against *Candida albican*s.

V. CONCLUSION

From the result obtained in this study, the following conclusions may be drawn;

- That the crude water, methanol and acetone extracts of the fruit husk of *P. biglobosa* contains very useful elements and bioactive constituents as phytochemicals
- That the *invitro* antibacterial and antifungal activities of the acetone and water extracts of the fruit husk of *Parkia biglobosa* showed that the extract had broad spectrum of activity against bacteria and are bactericidal and fungicidal in activity

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