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Original Article

Phytochemical and antimicrobial profile analysis of *Parkia biglobosa* against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Objective: The study was carried out to analyze the various phytochemical properties and to determine the *in-vitro* antimicrobial activity of *Parkia biglobosa* methanolic leaf extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA).
Materials and methods: The phytochemical screening and Thin Layer Chromatography (TLC) of the methanol extract were conducted using standard methods. The antimicrobial activity of the *P. biglobosa* methanolic leaf extract on sheep and human MRSA isolates was investigated by disc diffusion method. The extract was tested at different concentrations of 100, 200 and 400 mg/mL and norfloxacin (10 µg) as control. The MIC/MBC were determined by serial dilution method in broth media.
Results: the phytochemical analysis showed the presence of alkaloid, saponins, tannins, cardiac glycosides, flavonoids and terpenoids while TLC result revealed 5 spots with retention factor (Rf) values of 0.14, 0.34, 0.42, 0.56 and 0.66 depicting different component using methanol and chloroform at the ratio of 4:1 as solvent system. The diameter of inhibition zone (DIZ) of the extract on MRSA isolates cultured on agar plate revealed a dose dependent response. The extract had the highest DIZ average replicate value of 20.66±1.76 against human MRSA isolates and 20.66±1.20 was recorded as highest DIZ on the sheep MRSA isolates. Norfloxacin had the highest DIZ of 20.33±0.57 on sheep MRSA isolates and 20.33±1.52 DIZ against human isolates. MIC/MBC revealed the highest values of 6.25 mg/mL/12.5 mg/mL for both sheep and human MRSA isolates.
Conclusion: The susceptibility test of crude methanol extract of *P. biglobosa* leaf has revealed activity against MRSA. Thereby, attesting to the folkloric claim of using *P. biglobosa* for ethno-therapeutic purposes against infectious diseases in Nigeria and other places.

KEYWORDS

Antimicrobial; MRSA; Phytochemical

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INTRODUCTION

The increase in antibiotic resistance by microbes to almost all available antibiotics is a serious public health concern in the 21st century (Vadhana et al., 2015). In 2017 World Health Organization reported a plan to be applied globally on antimicrobial resistance state that "Antibacterial Agents in Clinical Development, an analysis of the antibacterial clinical development pipeline shows a serious lack of new antibiotics under development to combat the growing threat of antimicrobial resistance" (WHO, 2017). Majority of recently used drugs in the clinical records are not new in all respect but refitting of the classes of antimicrobial agents that are currently in used, and these can only give transient solutions. This will impede the ability of the biomedical scientist to control infectious diseases such as MRSA and it's like which have a detrimental effect on human and animal health with adverse effect on global trade and economy. However, it is difficult to envisage a world without antibiotics. But this is fast approaching because the diseases and disease-causing agents that were once believed to have been overcome and defeated by antibacterial agents are resurfacing in a different pattern of resistant to therapeutic agents (antibiotics) (Levy and Marshall, 2004).

However, human activities in both medical and veterinary sector facilitate emergence and widespread of antimicrobial resistance. Such activities include; improper therapeutic use of antimicrobial agents for human treatment, and their applications in agriculture, which includes; aquaculture, livestock and poultry and other forms of farming encourages the development and the select antibiotic resistance strain. In addition, inadequate control and prevention measures of infection practices contributes to the emergence and spread of antimicrobial resistance (Liu et al., 2016).

The fact that antimicrobial resistance is fast on the rise along with the development of the common antibiotics. It is therefore, essential to take crucial and harmonized measures to rescue the universe from entering the era of post antibiotic, where ordinary infection and minor injuries can be life threatening (Vadhana et al., 2015). In addition, there is an immediate need to develop a different line of drugs that can solve the menace of multidrug resistant microbes (Emeka et al., 2012). Presently, researchers are searching for molecules from plants that act as the cutting-edge antimicrobial which may possess a wider spectrum of activity against bacteria of both Gram-positive and Gram-negative origin and devoid of any serious side effects (Chandra et al., 2017). The ability of Plants to produce wide and infinite varieties of secondary metabolites forming the basis of a plant derived antimicrobial compounds. *Parkia biglobosa* (the African locust bean tree) is the member of the family Mimosaceae, order Leguminosae, and is native to Africa. It has been reported to contain some plant derived metabolites and the stem bark is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA) (Das et al., 2010; Jauro et al., 2016).

This study analyzed the various phytochemical properties and evaluated the *in-vitro* antimicrobial activity of *P. biglobosa* methanolic extract. The research was able to verify and confirmed the claims by people of Madagali Adamawa State, following the survey on the traditional application of various parts of the plant to treat ailments such as diarrhea, dental caries, pneumonia, otitis, bronchitis, wounds, conjunctivitis, dermatosis, tracheitis, haemorrhoids, and anti-inflammatory activities.

MATERIALS AND METHODS

Ethical Approval: The study was conducted using isolates obtained from the Veterinary Microbiology laboratory University of Maiduguri and the antimicrobial activity of the extract against MRSA was tested *in vitro*. Therefore, no ethical approval was requested for the study.

***P. biglobosa* Collection and authentication:** The plant leaves were collected from Dzuyel village in Madagali Local Government Area of Adamawa State, Nigeria. It was appropriately identified by a taxonomist at the Biological Science Department, University of Maiduguri, Nigeria and it was vouchered UM/PH/01/0011.

Preparation of plant and extraction: The collected parts of *P. biglobosa* were dried under shade, and then it was ground into powdered form using mortar and pestle before subjecting it to extraction by maceration in the Pharmaceutical Chemistry Department of Faculty of Pharmacy University of Maiduguri. 400 gm of the dried powdered leaves of *P. biglobosa* was soaked in 800 mL of 96% methanol followed by 24 h incubation at room temperature before extraction, this was redone three more times. The extract was filtered using No 2 filter paper (Whatman). The filtrate was dried by reduced pressure at 40°C using rotary evaporator.

Thin Layer Chromatography (TLC): The methanol extracts of the leaf of *P. biglobosa* was analysed using conventional thin layer chromatographic analyses method

according to Udobi and Onaolapo (2012) with some modifications. The TLC was carried out using silica gel pre-coated on aluminium plates (Merck, GF254, 20 X 20 cm, 0.2 mm thickness). 10 µL of the plant extracts was applied on TLC plate at equal distance with the help of micropipette and was developed with a mixture of ethanol/chloroform (4:1). The spots of the developed chromatogram were analysed using UV lamp fluorescent at 254 nm to visualized, followed by exposure to vanillin and sulfuric acid spray and heated to 100-115°C.

Phytochemical evaluation: The extract was analyzed using the following procedures of Talukdar et al. (2010) with few modifications, to qualitatively test for the inhabitance of saponins, alkaloids, flavonoids, tannins, terpenoids, reducing sugars, glycosides, , anthraquinones, volatile oils and phlobatannins.

Test for saponins: Saponins was detected using the froth test, by adding 1 gm of the plant extract to 10 mL of double distilled water, then boiled for 5 min in a conical flask followed by filtration. To 10 mL of distilled water in a test tube 2.5 mL of the filtrate was added. This was subjected to vigorous shaking for about 30 Sec with stoppered in place. It was then allowed to stand for half an hour. The presence of saponins is indicated by the honeycomb froth appearance.

Test for tannins: 3-4 drops of 10% ferric chloride was dropped to a diluted extract distilled water. The presence of gallic tannins was observed by blue color appearance and green color appearance shows the presence of catecholic tannins.

Test for reducing sugars: 0.5 mg of plant extract was dissolved in 1 mL of water followed by Fehling's solution (5-8 drops) then heated in a water bath. The presence of reducing sugars was shown by brick red colouration.

Test for glycosides: 5 mg of extract was dissolved in 25 mL of sulphuric acid in a test tube, this was then boiled for 15 min, NaOH (10%) was used to neutralized it after cooling, followed by 5 mL of Fehling solution. The appearance of precipitated brick red indicates the presence of Glycosides.

Test for alkaloids: 2 mg of the plant extract dissolved in picric acid. The alkaloid presence was by orange coloration .

Test for flavonoids: 1.5 mL of methanol (50%) was used to dissolve 4 mg of plant extract; it was then warmed, and then followed by addition of metal magnesium and concentrated hydrochloric acid 5-6 drops. The appearance of red color indicates the presence of flavonoids and orange color shows the presence of flavones.

Test for volatile oils: 2 mg of the plant extract was dissolved in sodium hydroxide (0.1 mL) and then diluted with small quantity hydrochloric acid then shacked. A white precipitate was formed as indication of a volatile oils present in the extract.

Test for terpenoids: Acetic anhydride (0.5 mL) and chloroform (0.5 mL) was used to dissolve 4 mg of plant extract. Followed by the addition of concentrated sulphuric acid slowly. The presence of terpenoid is shown by the appearance of red violet color.

Test for anthraquinones (Borntrager's reaction for free anthraquinones): The Borntrager's reaction method for testing anthraquinones was employed. 20 mL of chloroform was added to 1 gm of the methanolic crude plant extract in a test tube. This was followed by heating in a steam bath for 5 min. the extract was filtered while it was still hot and equal volume of ammonia (10%) added to the filtrate after cool. The presence of bright pink coloration at the upper aqueous top layer following shaking indicates Anthraquinones presence.

Test for phlobatannins (HCl test): The aqueous solution of the extract was diluted with 2 mL of Hydrochloric acid. The presence of red precipitate shows the presence of phlobatannins.

Preparation of extract concentration: The preparation of the stock solution of the various plant extracts was by dissolving 0.1, 0.2, and 0.4 gm in 1 mL double distilled water each to obtain the final concentration of 100, 200, and 400 mg/mL respectively, a standard antibiotic disc (Norfloxacin 10 µg, Oxoid Ltd. Basingstoke, Hamshire England) was used on all the microorganisms and their inhibition zone were measured and compared with those of extract.

Bacteriology: The methicillin-resistant *S. aureus* isolates from human and sheep used in this study were obtained from the Research Laboratory of the Veterinary Microbiology Department of the University of Maiduguri, Nigeria. They were phenotypically confirmed using conventional bacteriological methods such as colonial morphology (**Figure 1B**), characteristics of the Gram stain, catalase and coagulase tests. Isolates that were Gram-positive cocci on gram stain and catalase positive and coagulate human plasma were accepted to be *S. aureus* in addition to other standard biochemical test

(Cheesbrough, 2006; Kwoji et al., 2017). Cefoxitin (30 µg) susceptibility test and growth on ORSAB media (Oxoid Ltd. Basingstoke, Hamshire England) at the concentration of 2 mL of ORSAB supplement added to 50 mL of prepared ORSAB media as recommended by the manufacturer (**Figure 1C**). The isolates obtained from human and sheep were twelve each. The purified isolates propagation was done and stored on nutrient agar. The nutrient agar media were purchased from Oxoid, Ltd., England and the manufacturer's recommendation was followed to prepare the media. All the stock cultures were maintained at 4°C on nutrient agar plate and subcultured in nutrient broth (Oxoid, Ltd., England) at 37°C for 8 h prior to subjecting them to antibacterial test.

Sensitivity test of MRSA to methanol crude extract of the leaf: The antimicrobial activity of methanol extract of *P. biglobosa* leaf was tested against MRSA isolates confirmed at the Department of Veterinary Medicine Research laboratory. The methanol crude extract of *P. biglobosa* leaf was constituted at three different concentration of 400 mg/mL, 200 mg/mL and 100 mg/mL of distilled water, 25 discs of 6 mm in diameter prepared using filter paper No. 1 (Whatman) were impregnated into each working concentration to acquire the final concentration of 16 mg/mL/disc, 8 mg/ml/disc, and 4 mg/mL/disc respectively. Kirby-Bauer disc diffusion method was employ to carry out the antimicrobial test. Each isolate was constituted to McFarland turbidity standard 0.5 (containing approximately 10^6 CFU/mL) and cultured overnight, it was poured to cover the surface of the Mueller Hinton agar plates and excess was drained, allowed to dry while the lid of the Petri dish is in place. The standard antimicrobial discs that were impregnated into the extract working concentration were aseptically placed on the plates which have been already inoculated in triplicate and allowed to stand for about an hour. The plate incubation was done at 37°C for 18-24 h. The measurement of the diameter of the inhibition zone produced by each antimicrobial disc was done using ruler in millimeters.

Determination of Minimum Inhibitory Concentration (MIC): Micro-broth dilution method was used to determined Minimum inhibitory concentration, the crude extract was prepared by serial double dilution using distilled water where 1 gm of the crude plant extract was dissolved in 10 mL of double distilled water to obtain concentrations of 100 mg/ml, 5 mL was taken from the previous concentration and diluted with 5 mL of double distilled water in the second test tube to obtain the concentration of 50 mg/mL, and subsequently a serial dilution was done to obtain the following concentration; 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL. The prepared crude plant extract stock solution of various concentrations was then mixed with the inocula standardized with McFarland turbidity standard in test tubes. The tubes containing the crude plant extract and the inocula served as the test, whereas the tubes containing broth and crude extract served as control were incubated aerobically for 24 h at 37°C. 24 h post-incubation the minimum inhibitory concentration was determine by observing the lowest concentration without evidence of growth (Akinyemi et al., 2005)

Determination of Minimum Bactericidal Concentration (MBC): Nutrient agar plate was prepared and inoculated with the sample without affirmation of growth in the previous test (MIC). The plates were then incubated for 24 h at 37°C. The lowest dilution from MIC that were inoculated onto the Nutrient agar that yielded no evidence of colonial growth was regarded as MBC (Akinyemi et al., 2005).

Statistical analysis: The data generated from MRSA isolation and antimicrobial susceptibility test using conventional antibiotics and the crude extract of *P. biglobosa* leaf used in the research activity were analyzed using: Simple descriptive statistics (tables) and GraphPad Prism version 6.01 was used for one-way Analysis of variance (ANOVA). Value with $P<0.05$ is considered significant.

RESUTS

The percentage yield of the methanol leaf extract of *P. biglobosa* was 17.43% (69.72 gm) of 400 gm of powdered leaf materials used for extraction. The phytochemical evaluation of the crude methanol leaf extract of *P. biglobosa* showed that it contained alkaloid, saponins, tannins, cardiac glycosides, flavonoids and terpenoids whereas anthraquinones and phlobatannins were lacking in the crude plant extract as represented in **Table 1**. The result of thin layer chromatography (TLC) using methanol and chloroform in the ratio of 4:1 as developing solvent, revealed the presence of 5 spots with different Rf values presented in **Figure 1A** and **Table 2**. TLC profile of the crude plant extracts create discernment about a number of phytochemicals present through different Rf values in solvent system. This provides a very important clue in understanding the chemical constituent in the plant. It also helps to select

the solvent system which is appropriate for isolation of pure compounds using column chromatography.

Table 1. Phytochemical screening of methanol leaf extract of *Parkia biglobosa*

Chemical Compound test	Result
Alkaloids	+
Saponins	+
Tannins	+
Phlabotannins	–
Anthraquinones	–
Cardiac glycosides	+
Flavonoids	+
Terpenoids	+

+ = Present, – = Absent

Table 2. Spots of Retention factor (Rf) values of methanol leaf extract of *Parkia biglobosa*

Spots	Rf values
1	0.14
2	0.34
3	0.42
4	0.56
5	0.66

Rf=Retention factor, Developing solvent: Methanol and chloroform (4:1), Spray reagents used: Vanillin and sulfuric acid

The *in vitro* antimicrobial susceptibility test of MRSA to the methanol leaf extract of *P. biglobosa* at graded concentrations of 100, 200 and 400 mg/mL was tested against 24 MRSA isolates, 12 each from humans and sheep is presented in **Table 3** and **Figure 1B**. The extract exhibited the highest diameter of the zone of inhibition (DZI) of 20.66±1.76 at concentration of 400 mg/mL against human isolate (H11) and in the animal isolates had DZI of 20.66±1.20 against isolates S8 and S9 at 400 mg/mL. The lowest value of 10.66±0.88 at 100 mg/mL was recorded for human isolates H3 and 9.00±1.52 for the sheep isolates S1 at 100 mg/mL. The DZI value the standard drug (Norfloxacin 10 µg) had against the isolates from both humans and sheep showed no significant difference with those produced by the *P. biglobosa* leaf extract at the highest concentration of 400mg/ml. The results obtained in **Table 3** is the average of three replicates of the various concentrations used in the experiment as shown in **Figure 1D**.

Table 4 shows the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanol leaf extract of *P. biglobosa* against MRSA isolated from human. The extract exhibited the highest MIC values of 6.25 mg/mL against isolates: H4, H10 and H11 while the highest MBC value were recorded for isolates: H1, H4 and H8 at the concentration of 12.5 mg/mL, with the lowest MIC value of 25 mg/ml observed against isolates H6, H9 and H12 and the lowest MBC value of 50 mg/mL were observed for isolates H6 and H9. Whereas, MIC and MBC of the methanol leaf extract of *P. biglobosa* against MRSA isolated from sheep are shown in **Table 5**. The leaf extract of *P. biglobosa* exhibit the highest MIC value of 6.25 mg/mL for S5 and S6 isolates and the lowest value at 25 mg/mL against S1, S3, S7, S9 and S11 isolates. The MBC value of 12.5 mg/mL recorded for S2, S6, S8 and S10 isolates respectively while the lowest MBC value of 50mg/ml was observed for S1, S7 and S11 isolates in that order.

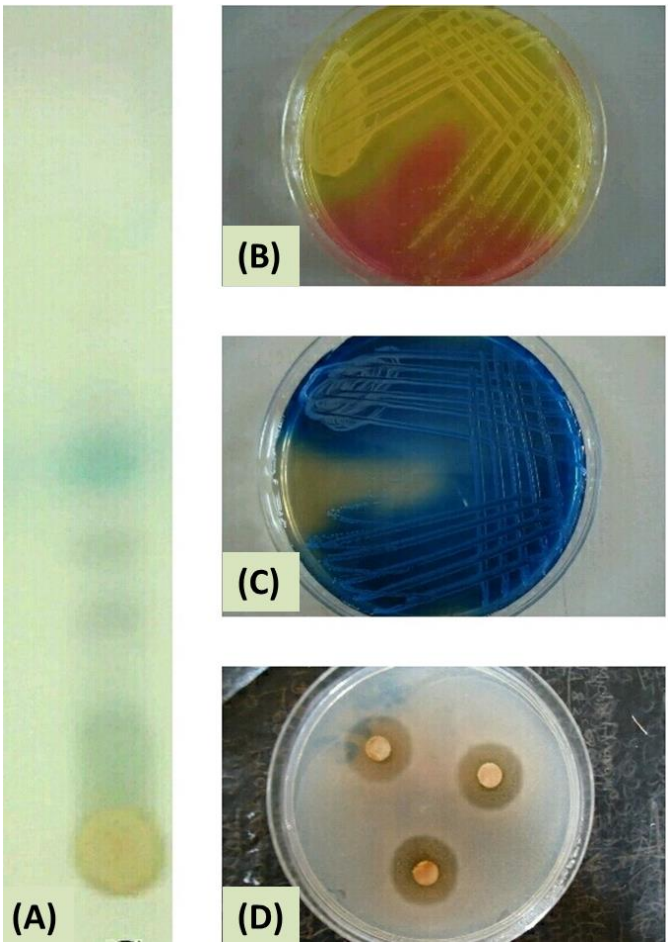

Figure 1. (A) Thin Layer Chromatography of methanol extract of *Parkia biglobosa* leaf using Ethanol/Chloroform (4:1) as developing solvent and vanillin and sulfuric acid as spray reagent. (B) Culture plate showing golden yellow coloration of *S. aureus* on mannitol salt agar. (C) Culture plate confirming MRSA on ORSAB media by producing deep blue color. (D) Culture plates showing the zone of inhibition produced by methanol leaf extract of *Parkia biglobosa* against MRSA isolates on Muller Hinton agar

Table 3. Susceptibility test of the methanol extract of *P.biglobosa* leaf against MRSA isolated from sheep and humans

Isolates	Extract (mg/mL)	MRSA isolates											
		1	2	3	4	5	6	7	8	9	10	11	12
Humans	100	15.66±0.88	14.00±0.00	10.66±0.88	13.33±0.33	15.00±1.00	11.66±0.88	14.00±0.00	13.33±1.33	11.66±0.66	11.66±1.20	15.66±1.33	13.66±0.66
	200	17.33±1.20	16.00±0.57	12.66±0.88	15.00±0.57	17.66±0.66	14.33±0.66	17.00±0.57	16.33±0.33	14.33±1.76	15.33±0.33	17.66±1.45	17.00±1.52
	400	19.33±1.76	19.33±1.85	15.00±0.57	19.00±1.00	19.33±0.88	17.00±1.52	19.66±0.33	19.33±1.33	18.00±0.00	18.33±1.20	20.66*±1.76	19.00±2.08
	Control	17.33±0.57	17.00±2.00	16.33±1.52	16.66±0.57	17.00±0.00	16.33±0.57	0.00±0.00	20.33±0.57	0.00±0.00	20.33±0,57	18.00±1.00	15.00±0.00
Sheep	100	9.00±1.52	12.00±0.57	14.33±0.33	13.33±1.20	11.33±0.88	11.00±01.52	10.00±0.57	10.33±0.33	16.33±1.85	12.33±0.66	13.66±1.45	14.00±0.00
	200	11.00±0.00	15.33±0.33	17.00±1.52	17.00±0.57	14.66±0.66	13.33±1.20	13.66±0.66	14.00±0.57	18.33±1.45	16.66±0.33	15.66±2.18	17.66±1.20
	400	14.66±0.88	18.66±0.88	20.00±1.00	19.00±1.52	17.66±0.33	17.00±0.00	18.33±1.45	20.66±1.20	20.66*±1.20	18.66±0.66	17.33±1.85	20.00±1.52
	Control	17.33±0.57	18.00±1.00	17.33±0.57	17.00±0.00	18.33±0.57	15.66±0.57	17.66±0.57	18.00±1.73	17.00±0.00	17.33±0.57	20.33±1.52	20.33±0.57

Control = Norfloxacin (10µg), 1-12=number of isolates

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extract of *P. biglobosa* leaf on MRSA isolated from Humans

Extracts (mg/mL)	Human MRSA isolates																							
	H1		H2		H3		H4		H5		H6		H7		H8		H9		H10		H11		H12	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3.125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	β	+	+	+	+	+	+	+	+	+	+	+	β	+	β	+	+	+
12.5	β	δ	β	+	β	+	β	δ	β	+	+	+	β	+	β	δ	+	+	-	+	-	+	+	+
25	-	-	-	δ	-	δ	-	-	-	δ	β	+	-	δ	-	-	β	+	-	δ	-	δ	β	δ
50	-	-	-	-	-	-	-	-	-	-	-	δ	-	-	-	-	-	δ	-	-	-	-	-	-

β=MIC, δ=MBC, +=Presence of growth, - =Absence of growth, H1-H12=Number isolates

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extract of *P. biglobosa* leaf on MRSA isolated from Sheep

Extracts (mg/mL)	Sheep MRSA isolates																							
	S1		S2		S3		S4		S5		S6		S7		S8		S9		S10		S11		S12	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3,125	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.25	+	+	+	+	+	+	+	+	β	+	β	+	+	+	+	+	+	+	+	+	+	+	+	+
12.5	+	+	β	δ	+	+	β	+	-	+	-	δ	+	+	β	δ	+	+	β	δ	+	+	β	+
25	B	+	-	-	β	δ	-	δ	-	δ	-	-	β	+	-	-	β	δ	-	-	β	+	-	δ
50	-	δ	-	-	-	-	-	-	-	-	-	-	-	δ	-	-	-	-	-	-	-	δ	-	-

β = MIC, δ = MBC, +=Presence of growth, - =Absence of growth, S1-S12=Number isolates

DISCUSSION

The antimicrobial activity of *P. biglobosa* leaf methanol extract shows concentration dependent pattern on the MRSA isolates. The variabilities were similar to the previous work on *P. biglobosa* methanol leaf extract by Obajuluwa et al. (2013) he reported the extract has activity on MRSA isolated from human skin and hospital bed side. Ajaiyeoba (2002) also reported that ethanolic leaf extract of *P. biglobosa* showed antibacterial activity against MRSA, these findings are also supported by Hall et al. (1997). Udobi et al. (2010) findings which revealed susceptibility of *S. aureus* to the stem bark methanolic extract of *P. biglobosa*. The results from this study have confirmed the claims by folklore medicine of the use of *P. biglobosa* for the treatment of infections such as tracheitis, dental caries, bronchitis, pneumonia, diarrhoea, wounds, conjunctivitis, otitis, dermatosis and haemorrhoids.

Four hundred gram (400 gm) of powdered leaf materials was used for extraction and, 69.72 gm (17.43%) was obtained from the crude extract of methanol leaf extract of *P. biglobosa*. The evaluation of the phytochemicals present in *P. biglobosa* methanol leaf extract showed the presence of tannins, flavonoids, saponins, glycosides, cardiac, alkaloids and terpenoids. The TLC result revealed the presence of many spots in this study (**Table 2**). The antibacterial activity observed in this study could be associated with some of these secondary metabolites detected in the *P. biglobosa* methanol leaf extract, because this concur with report by Jauro et al. (2016) where he reported similar secondary metabolites in aqueous extract of *P. biglobosa* stem bark.

Flavan-3-ol (flavonoids) compounds 8-[1-(3,4-dihydroxyphenyl)-3-methoxy-3-oxopropyl]-catechin is one of the chemical substances isolated from the plant and it possessed antibacterial activity (Zhang et al., 2013), this supports Abagale et al. (2013) who reported the present flavan-3-ol compounds in *P. biglobosa*. Flavonoids has showed some extensive amount activities biologically these include; antimicrobial, anti-angionic, anti-inflammatory, analgesic, cytostatic, anti-allergic effects, and antioxidant properties. Plants are known for their ability to synthesize flavonoids to respond to microbial infection (Al-Bayati and Al-Mola, 2008). Flavonoids have been reported to form complexes in the bacterial cell both extracellular and in soluble proteins which is believed to be flavonoids mechanism of action on microbes (Scalbert, 1991; Musa et al., 2008). These further support the claims that *P. biglobosa* have been utilized for folklore remedies for many diseases in Africa. Tannins have been reported to act by interfering with the biological activity via interacting with certain proteins which include the cellular envelopes, complex forming polysaccharide and deprive the bacteria certain irons and also interfere with the hydrogen bond (Scalbert, 1991; Akiyama et al., 2001), these results in nutritional deficiency for the microorganism due to microbial protein precipitation (Ogunleye and Ibitoye, 2003). Tannins in herbs act as the astringent in nature, and this is known to be helpful in intestinal disorders, especially diarrhoea and dysentery (Dharmananda, 2003) thereby, revealing *P. biglobosa* extract's antimicrobial activity by inhibits the growth of bacteria pathogen. Hence, supports why the Yoruba tribe of South-western Nigeria use this plant for the treatment diarrhea and dysentery (Dharmananda, 2003). This concur with the report by Millogo-Kone et al. (2006) where he reported that trans-ferulic acid, long chain cis-ferulates (2a-e) and lupeol as the constituent of tannic acid, phenolic compounds of condensed tannin are known to have antibacterial activity. The antibacterial activity seen in the crude extract in this work may be related to the presence of saponin compounds in *P. biglobosa* secondary metabolite components such as 3-*O*-(2'-acetamido-2'-deoxy-β-D-glycopyranosyl) olean-12-en-28-oic acid (aridanin) and 3-*O*-[β-D-glucopyranosyl-(1"-3')-2'-acetamido-2'-deoxy-β-galactopyranosyl]olean-12-en-28-oic acid (lotoidoside E) as reported by Lunga et al. (2014). Saponins' mechanism of actions such leakage of proteins and enzymes have been linked to its ability to initiate cell lysis in bacteria (Al-Bayati and Al-Mola, 2008) it was also been reported by Just et al. (1998) that saponins has anti-inflammation. Alkaloids are other secondary metabolites reported in this study that researchers have reported to be associated with antibacterial properties because of their DNA intercalating ability (Phillipson and O'Neill, 1984). The antibacterial activity recorded in this study could be associated with some of these secondary metabolites detected in the *P. biglobosa* methanol leaf extract.

CONCLUSION

The antimicrobial and phytochemical screening of the crude methanol extract of *P. biglobosa* leaf revealed that it contains phytochemical compounds that are known to possess antibacterial properties. The extract has shown promising activity at the MIC/MBC concentration of 6.25 mg/mL and 12.5 mg/mL as well as the diameter of the zone of inhibition for both sheep and human MRSA isolates. The results obtained from antibacterial activity of the crude extract of *P. biglobosa* gives high hopes for

unfolding a new antimicrobial agent to regulate and restraint MRSA menace. *P. biglobosa* can be an alternative antibacterial source for treating multi-drug resistant bacteria. Based on the findings in this study, the following recommendations were made: More research should be carried out on *P. biglobosa* to isolate bioactive pure compounds, elucidate their structures and evaluate their anti-MRSA activities by determining the toxicity and their safety in laboratory animals model and the mechanism of action against MRSA.

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CONFLICT OF INTEREST

No potential conflicts of interest that was declared by the authors with respect to authorship, the research, and/or publication of this article.

AUTHORS' CONTRIBUTION

SJ, MBA, YAG and II designed the study. SJ and MYZ collected the plant samples. The laboratory works were conducted by SJ, IAG, IDK and II. The manuscript was prepared by SJ and II, then reviewed by all the authors

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