

## Antimicrobial Susceptibility Test of Aqueous Extract of *Parkia biglobosa* Stem Bark on Methicillin Resistant *Staphylococcus aureus* (MRSA)

Solomon Jauro<sup>1\*</sup>, Mohammed Yusuf Zanna<sup>1</sup>, Fatima Adamu Lawan<sup>1</sup>,  
Istifanus James Waziri<sup>3</sup>, Isa Adamu Gulani<sup>2</sup>, Mustapha Bala Abubakar<sup>1</sup>.

<sup>1</sup>Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria.

<sup>2</sup>Department of Veterinary Medicine, University of Maiduguri, Nigeria.

<sup>3</sup>Nigeria Field Epidemiology and Laboratory Training Program.

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**Abstract:** The study into the phytochemical constituents and the in-vitro antibacterial effects of the crude aqueous extract of *Parkia biglobosa* was evaluated against twelve (12) Methicillin-resistant *Staphylococcus aureus* (MRSA) using agar plate disc diffusion technique at varying concentration of 100, 200, 400 and 600mg/ml with vancomycin 10µg standard drug as control. Preliminary phytochemical screening test of the aqueous extract revealed the presence of the following secondary metabolites; saponins, terpenoids, garlic tannins, reducing sugar, alkaloids, and flavonoids. The susceptibility of MRSA was tested against MRSA isolates from Human and Sheep. The diameter of zone of inhibition (DZI) on plates cultured with MRSA isolate revealed a dose dependant increase with the highest DZI of 25.33±1.33 on sheep MRSA isolate and 30.00±1.52 on human MRSA isolates, whereas the standard drug used as control (Vancomycin 10µg) had the highest DZI value of 20.33±0.88 against both humans and sheep MRSA isolates. The MIC/MBC of both sheep and humans MRSA isolates were investigated by micro-broth dilution method and result revealed the highest MIC/MBC values of 12.50mg/ml/25.00mg/ml of the extract against sheep MRSA isolates and 6.25mg/ml/6.25mg/ml of the extract against human MRSA isolates. The susceptibility pattern and MIC/MBC of crude aqueous extract of *Parkia biglobosa* stem bark, when compare with the values generated on the standard drugs used in this study. The result have revealed that the plant is effective against MRSA, and this have further confirmed the traditional claimes of the medicinal uses different parts of the plant to treat infectious and non-infectious diseases in Nigeria and beyond.

**Keywords:** antimicrobial, *Parkia biglobosa*, Methicillin, resistant, *Staphylococcus aureus* (MRSA)

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### I. Introduction:

Antibiotic resistance is the ability of a microorganism to prevail against the effects of an antibiotic. The extensive use of antibiotics over the last 50 years has led to the emergence of bacterial resistance and to the dissemination of resistance genes among pathogenic microorganisms. *Staphylococcus aureus* is one of the most important pathogens that can cause suppuration, abscess formation, a variety of pyogenic infection and even fatal septicemia in humans and animals. Methicillin-resistant *Staphylococcus aureus* (MRSA) is still considered as an emerging pathogen and public health threats result from the spread of hospital-acquired as well as community-acquired MRSA and livestock associated MRSA (1).

Over the past decades, MRSA has spread throughout the world and has become highly endemic in many geographic areas (2). MRSA infections are difficult to treat because of their resistance to many of the commonly used antibiotics which are not only to β-lactams and synthetic penicillin but also to macrolides, tetracyclines and aminoglycosides. Some of these MRSA strains are resistant to even the most powerful antibiotics, including vancomycin (3,4,5). WHO has acknowledged the need to identify new antibiotics and/or new approaches to overcome the growing problems associated with such infectious agents.

Medicinal plants are invaluable therapeutic resources (6,7). The used of plant-derived compounds in treatment of infections is an ancient practice that is employed throughout the world, especially in developing countries where traditional medicines are used to treat a variety of diseases. Interest in plants with antimicrobial properties has been revived as a result of current resistance profiles associated with over and inappropriate use of antibiotics (8).

*Parkia biglobosa* serves as a remedy for number of ailments; decoction of the stem bark is used as a mouthwash to steam and relieve toothache as well as a bath for fever. Irvine (9)(1961) reported that bark is used with lemon against wounds and ulcers. In cote d Ivoire and Nigeria, the stem bark infusion is used as a tonic against diarrhea and also as an enema (10). *Parkia biglobosa* has found so much medicinal use among the Hausa people of Northern Nigeria. It is used against bronchitis, pneumonia, diarrhea, violent colic, vomiting sores and ulcers. The leaves are also used for toothaches as well as for sore eyes in Gambia (11). The root of

*Parkia biglobosa* has been reported to be used in lotions for sore eyes when combined with leaves. They are also known to be active against bronchitis, pile, cough, amoebiasis, dental carries and conjunctivitis. (12).

This work was conducted to investigate and evaluate the antimicrobial activities of the plant claimed in folklore medicine and the phytochemical compounds present in the plant.

## **II. Materials And Methods**

### **2.1 Sample Collection and Identification**

The stem bark of *Parkia biglobosa* was collected from Madagali Local Government Area of Adamawa State. It was then submitted to the Department of Biological Science, Faculty of Science, University of Maiduguri, Nigeria for identification.

### **2.2 Preparation of Plant Extract**

The collected stem bark of *Parkia biglobosa* was dried under shade, and then grinded into powdered form using pestle and mortar before subjecting it to extraction in Drugs Laboratory of NAFDAC.

#### **2.2.1 Aqueous Extraction**

To 100g of the dried powdered stem bark of *Parkia biglobosa*, 250ml of distilled water was added. It was thoroughly mixed and allowed to stand for 1 hour. The extract was separated using sterile muslin cloth then filter through sterile Whatman filter paper No.2. Hot air oven at 45°C was used to dry the filtrate (13).

### **2.3 Phytochemical Analysis**

The extracts were analyzed using the procedures of Takukdar et al., (14), to test for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

### **2.4 Preparation of Extract Concentration**

Stock of solution of the plant extracts was prepared by dissolving 0.1, 0.2, 0.4 and 0.6g in 1 ml distilled water each to obtain a concentration of 100, 200, 400 and 600mg/ml respectively, a standard antibiotic disc (Vancomycin 10µg, Oxoid Ltd. Basingstoke, Hampshire England) was used on all the organisms and their zone of inhibition were compared with those of extract.

### **2.5 Bacteriology**

Twelve (12) pure laboratory isolates of methicillin-resistant *Staphylococcus aureus* were obtained from the department of Veterinary Medicine Research Laboratory, University of Maiduguri, Nigeria. They were phenotypically identified using established bacteriological methods that include colonial morphology, Gram stain characteristics and catalase and coagulase tests (15). Isolates that were Gram-positive cocci, catalase positive and coagulated human plasma were considered as *S. aureus* in addition to other standard biochemical test (16). Cefoxitin susceptibility test and growth on ORSAB media (Oxoid Ltd. Basingstoke, Hampshire England) at the concentration of 2ml of ORSAB supplement added to 50ml of prepared ORSAB media as recommended by the manufacturer. Six of the isolates were obtained from human and sheep each. The purified isolates were propagated and stored on nutrient agar plates. The nutrient agar media were obtained from Oxoid, Ltd., England and were prepared according to the manufacturer's recommendation. All stock culture were maintained in nutrient agar plate at 4°C and subcultured in nutrient broths (Oxoid, Ltd., England) at 37°C for 8 hours prior to antibacterial testing.

### **2.6 Antimicrobial Susceptibility Test**

The crude aqueous extract of *Parkia biglobosa* stem bark were subjected to preliminary antimicrobial evaluation on twelve methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from humans and sheep at the Department of Veterinary Medicine Research Laboratory using disc diffusion methods in accordance with the Clinical Laboratory Standard institute (17). Four different working concentrations of the extract were constituted at 100, 200, 400 and 600mg/ml of distilled water and, 25 discs of 6mm in diameter prepared using whatman filter paper No. 1 were impregnated into the working concentrations, this gave the concentration of 40, 80, 160, and 240µg/disc respectively. The overnight cultures of the bacterial isolates were diluted using sterile normal saline to give an inoculum size of about 10<sup>8</sup> cfu/ml according to McFarland turbidity standard. About 0.5ml of the diluted culture was aseptically dispensed on the surface of sterile petri-dishes containing sterile solid nutrient agar. Discs containing the plant extract were aseptically mounted on agar plate in triplicates and incubated at 37°C for 24hours. The disc containing Vancomycin 10 µg/disc was used as positive control. Diameter of zone of inhibition ≥ 10 mm produced by the extracts are considered active (18) and were compared to that of the standard antibiotics. The inhibition zones observed were recorded in millimeter using a transparent meter-ruler.

### 2.7 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using nutrient broth dilution technique as described by Vallekobia et al., (19). The stock extracts concentration of 100mg/ml was made by dissolving 1g of the extract in 10ml of sterile distilled water and the working concentration prepared by two fold serial dilution technique that range from range 50, 25, 12.5, 6.25 and 3.125mg/ml respectively. The test organism (0.5ml) was pipette into each of the test tubes and incubated at 37°C for 24hours. The lowest concentration where no evidence of growth is seen determined as the MIC.

### 2.8 Determination of Maximum Bactericidal Concentration (MBC)

The MBC was determined using the broth dilution technique by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37°C for 24hours for possible bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered as the minimum bactericidal concentration (19).

### 2.9 Statistical Analysis

The data to be generated from the study was analysed using SPSS version 16.0 to run analysis of variance (ANOVA) and the result of the antimicrobial activity of the plant extracts was expressed as mean standard error using same statistical software.

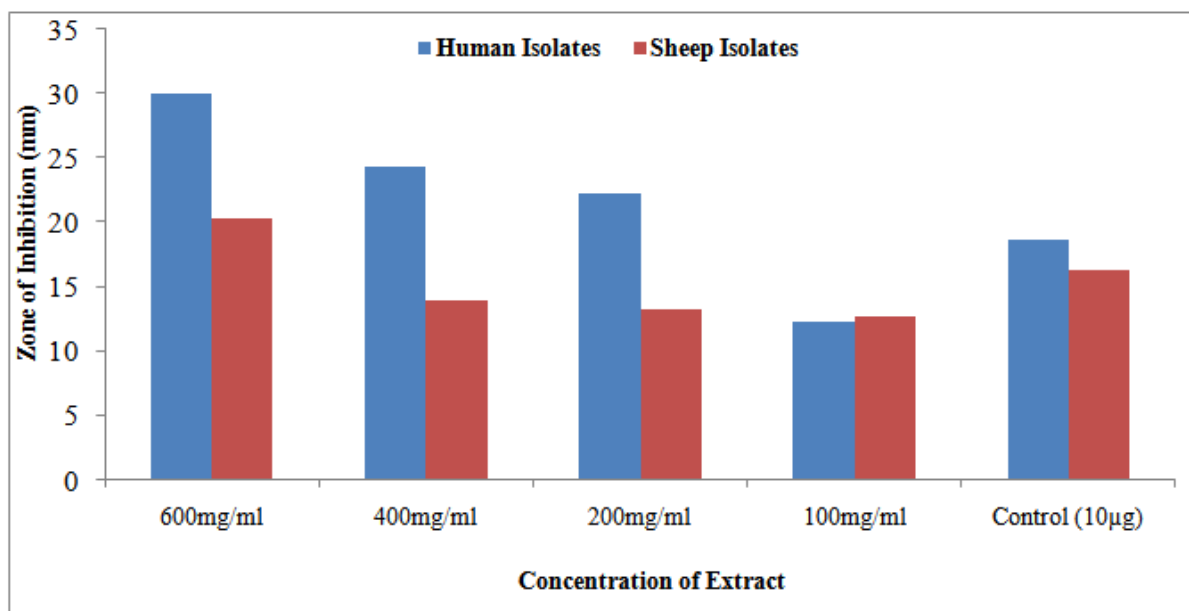
## III. Result

The phytochemical analysis of aqueous crude extract of *Parkia biglobosa* stem bark showed the presence of the following secondary metabolites: saponnins, terpenoids, galic tannins, reducing sugar, Alkaloids and flavonoid, but it does not contain glycosides and volatile oil in table 1. The antibacterial activity of the ethanol extract of *Parkia biglobosa* stem bark is assessed by the diameter of zone of inhibition is shown in figure 1. They showed strong antibacterial activity against MRSA of Human and animal origin used in this study. MRSA of both origin showed very good susceptibility to the extract at the concentration of 600mg/ml, with human isolates showing the highest susceptibility with outstanding zone of inhibition of 30mm in diameter, in the same vein human MRSA isolates exposed to the extract at the concentration 400 mg/ml and 200 mg/ml showed high susceptibility with diameter of zone of inhibition ( $\geq 20$ mm) but MRSA isolate from sheep showed 14mm in diameter at 400 mg/ml and 13.33 mm in diameter at 200 mg/ml. This is followed by the lowest diameter of zone of inhibition 12.66 and 12.33mm in diameter recorded against MRSA from human and sheep at the concentration of 100 mg/ml. The standard antibiotic (control) that was used produced DZI of 18.66 mm in diameter against human MRSA isolates and 16.33 mm in diameter against MRSA of sheep. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanol extract of *Parkia biglobosa* stem bark against MRSA isolated from human and sheep are presented in table 2. The extract showed MIC value of 6.25mg/ml against MRSA of human and 12.50mg/ml as MIC value against MRSA sheep origin. Whereas MBC value of 6.25mg/ml was observed against MRSA isolated from human and 25.00mg/ml was recorded against MRSA isolated from sheep.

**Table 1: Phytochemical Analysis of aqueous crude extract of *Parkia biglobosa* stem bark**

S/N	Compounds	Ethanol Extract
1	Saponnins	+
2	Terpenois	+
3	Garlic tannin	+
4	Reducing Sugar	+
5	Glycosides	-
6	Alkaloids	+
7	Flavonoid	+
8	Volatile oil	-

Key: + = Present, - = Absent



**Figure 1:** Zone of Inhibition of Aqueous Extract of *Parkia biglobosa* Stem Bark against MRSA

**Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol Extract of *Parkia biglobosa* Stem Bark against MRSA**

Concn. Of extract	Human Isolates		Sheep Isolates	
	MIC	MBC	MIC	MBC
100mg/ml	-	-	-	-
50mg/ml	-	-	-	-
25mg/ml	-	-	-	$\beta$
12.5mg/ml	-	-	$\alpha$	+
6.25mg/ml	$\alpha$	$\beta$	+	+
3.125mg/ml	+	+	+	+

Key:  $\alpha$  = MIC,  $\beta$  = MBC, + = Presence of MRSA growth, - = Absence of MRSA growth.

#### IV. Discussion

The result of antimicrobial activity of aqueous extract of *Parkia biglobosa* stem bark shows concentration dependence response, with gross and broad spectrum activity against the tested MRSA isolates. There was no significant difference in susceptibility between the MRSA isolates of human and sheep origin as shown in figure 1. The result obtained in this study were in some respect similar to some work done in the past on the antibacterial activity of *Parkia biglobosa* stem bark aqueous fraction by Udubi *et al.*, (20). The bioautographic activity of the plant was reaffirm by Udobi *et al.*, (21) when he reported; the plant *Parkia biglobosa* have good activity individually against *S. aureus*. The finding of this study is in agreement with the report by Obajuluwa *et al.*, (22); Ajaiyeoba (23) and Hall *et al.*, (24) where they reported that the extracts of *Parkia biglobosa* have excellent antibacterial activity against MRSA.

The preliminary phytochemical screening of aqueous extract of *Parkia biglobosa* stem bark shows the presence of secondary metabolites which includes; flavones, garlic tannins, saponins, cardiac glycosides, terpenoids, volatile oil and alkaloids. Udobi *et al.*, (20) have reported similar phytochemical compound with the exception of alkaloids in his study on *Parkia biglobosa* stem bark, he reconfirm the phytochemical constituent in 2012 when he analyzed aqueous extract of *Parkia biglobosa* stem bark. The result of this study is also consistent with the result obtained by Obajuluwa *et al.*, (22) when he reported phytochemical and antimicrobial properties of methanolic extract of *Parkia biglobosa* root, stem bark and leaf. Also the results obtained in this study are in accordance with the findings of by Hall *et al.*, (24). It has been established that high presences of flavonoids, tannins and saponins, which are secondary metabolites in *Parkia biglobosa* have contributed to it antibacterial activities (23). The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins (25). Also, the crude extract of *Parkia biglobosa* root bark contains saponins, glycosides, tannins and a trace of alkaloids (26). Saponins acts on bacteria by causing cell membrane lysis leading to leakage of proteins and certain enzymes from bacterial cells (27). Phillipson and O'Neill (28) reported that Alkaloids exhibits antibacterial properties/activities by intercalating with bacterial DNA. Since the presence of these metabolites in plants have been linked to the antimicrobial activities of the plants(29, 25).

## V. Conclusion

Based on this study, it has been concluded that the crude aqueous extract of *Parkia biglobosa* contains phytochemical compounds that are known possess antibacterial properties. The results obtained from antibacterial activity of the crude extract of *Parkia biglobosa* gives high hopes for the development of a new agent for the control of MRSA. Which will be an alternative antibacterial source for treating multi-drugs resistant bacteria.

## VI. Recommendations

Based on the finding of these studies, it is therefore recommended that further studies should be carried out on the two plants to identify the active principles responsible for their antibacterial activity. Mechanism of action of *Parkia biglobosa* against MRSA should also be determined Toxicities studies should be carried out on *Parkia biglobosa* to determine their safety in animal models and *in-vivo* studies should be carried out on *Parkia biglobosa* to determine their activities in laboratory animals.

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