

**COMPARATIVE STUDIES OF THE ANTIBACTERIAL ACTIVITIES OF THE EXTRACTS OF PARTS OF THE AFRICAN LOCUST BEAN (PARKIA BIGLOBOSA) TREE AGAINST HYPER BETA LACTAMASE PRODUCING STAPHYLOCOCCI (PHENOTYPIC MRSA) ISOLATES FROM ORTHOPAEDIC PATIENTS.**

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## **ABSTRACT**

The antibacterial effect of the methanolic extracts of the leaves, stem bark and root of the African Locust Bean tree (*Parkia biglobosa*) against methicillin resistant *Staphylococcus aureus* (MRSA) isolated from the wounds, skins and beds of orthopaedic patients was studied and compared. This was with the intention of developing an alternative source of antibacterial agent that can be used against these resistant organisms from this plant. A total of 33, 36 and 48 isolates of hyper beta lactamase producing Staphylococci ( MRSA) from wounds, skin and beds of these patients were subjected to antibacterial activity test using the agar diffusion method. Results obtained showed that the crude methanolic extracts of the stem bark of *Parkia biglobosa* has the best activity followed by the roots and the leaves with these activity being concentration dependent. These results show that if the active constituents in the stem bark of *Parkia biglobosa* are identified, they can be compounded into a compatible dosage form that may serve as an alternative therapy for infections due to MRSA.

## **KEYWORDS**

Antibacterial, Resistant, Methanolic, Activity, Therapy, Methicillin resistant.

## **INTRODUCTION**

At the time of the discovery of antibiotics as chemotherapeutic agents, it was believed that infectious diseases would be eradicated.

These diseases are however returning in new forms that are resistant to antibiotic therapies<sup>4</sup>. This is due to an increasing frequency of drug resistance which has been attributed to a combination of

microbial characteristics, selection pressure due to antimicrobial use and societal and technological changes that enhance the transmission of drug resistant organisms<sup>7</sup>. The need for alternative sources of antimicrobial agents which can be used to control disease situations due to these organisms has continued to arise and medicinal plants have recently shown great potentials. Since the discovery that some plants contain compounds that can inhibit the growth of microorganisms, man has 'invaded' the plant kingdom searching continuously for those plants. Actually, before the onset of the synthetic era, man was entirely dependent on medicinal plants for the prevention and treatment of diseases. With the onset of research in medicine, it has been concluded that plants contain active principles which are responsible for their curative actions.

*Parkia biglobosa* (Jacq) Benth popularly called the African Locust bean tree is a multipurpose tree used for a wide range of purposes especially in the West African region. It grows up to a height ranging from 7m to 20 m. The leaves are alternate, dark green and bipinnate with about 13 to 60 pairs of leaflets. The stem bark is dark grayish-brown, thick and fissured. It has a deep taproot system which gives it the capacity to withstand drought conditions. (Agroforestry Database). *Parkia biglobosa* seeds are fermented into Dawadawa (daddawa) which is used for seasoning traditional soups in the West African sub-region. Other parts of the plant are also used for a wide range of other purposes e.g the branches are used as timbre and fuel. The fibre from the pods and root are used as sponges and as strips for musical instruments. The most pronounced uses of parts of this plant however, are medicinal e.g the bark is used as a mouth wash, vapour inhalant for toothache or for ear complaints. It is also marcerated in baths for leprosy and used for bronchitis,

pneumonia, diarrhea and violent colic. The leaves are used in lotions for sore eyes, burns and toothache while the roots are used in lotion for sore eyes.

Methicillin Resistance *Staphylococcus aureus* (MRSA) are isolates of *Staphylococcus aureus* which have acquired genes encoding antibiotic resistance to all penicillins including methicillin<sup>3</sup>. Some *Staphylococcal* isolates however, have been found to be mec A negative in the polymerase chain reaction (PCR) but resistant to methicillin and oxacillin. This has been attributed to other mechanisms like the hyper production of Beta lactamase which may have conferred the methicillin resistance ability on these organisms. The isolates used for this work are those kind of *Staphylococcus aureus*

The results presented in this report do not only confirm these *Parkia biglobosa* parts as having good antibacterial potentials but goes further to compare their efficacies against the problematic 'superbug'- Methicillin resistant *Staphylococcus aureus* isolated from orthopaedic patients in the Ahmadu Bello University, Zaria, Nigeria.

## EXPERIMENTAL

### Media Preparations

All media used were reconstituted according to the manufacturer's specifications, sterilized at 121<sup>0</sup>c and stored at 4<sup>0</sup>c until required.

### Isolation and Purification of *Staphylococcus aureus*

Organisms used were isolated from the wounds, beds and skins of orthopaedic patients using sterile cotton swabs moistened in sterile peptone water. The swab was firmly applied, slowly rotated

thoroughly covering the surface of the wound and or fractured area. The same was done for patients beddings and skin. The swab was then dropped in a sterile nutrient broth, placed in an ice pack and taken to the laboratory to be incubated at 37<sup>0</sup>c for 18 hours. Observed colonies were further screened for methicillin resistance ability and stored in the refrigerator at 4<sup>0</sup>c on nutrient agar slants.

## **DETECTION OF MRSA**

This detection was done using a Clinical laboratory standard institute (CLSI) formerly NCCLS guideline. *Staphylococcus aureus* isolates were spot inoculated unto Mueller Hinton agar supplemented with 6µg/ml oxacillin and 4% sodium chloride from a 0.5 Mcfarland standard suspension. The plates were incubated at 35<sup>0</sup>c for 24 hours. Isolates which grew showing more than one colony were considered methicillin resistant.

### **Collection and authentication of *Parkia biglobosa***

The plant materials (leaf, stem bark and root) were collected from Samaru- Zaria in Kaduna state, Nigeria. They were authenticated in the herbarium section of the Biological Sciences Department of Ahmadu Bello University Zaria where it is preserved with the voucher number 2846.

### **Preparation and Extraction of Samples**

The plant samples were air dried and ground into powder in a mortar. They were then each extracted to exhaustion with methanol using

a soxhlet apparatus. The solvent was thereafter removed and the yield (extract) was stored in the dessicator until needed.

### **Antibacterial activity of plant parts against MRSA**

An overnight broth culture of each isolate was used to seed sterile molten Mueller Hinton agar medium maintained at 45<sup>0</sup>c. They were allowed to set and wells 6mm in diameter were made on them using sterile standard cork borer. Various concentrations of the plant extract ranging from (10mg/ml-25mg/ml) were added to each well. The plates were allowed to stand at room temperature for one hour to allow for pre-diffusion and thereafter incubated at 37<sup>0</sup>c for 24 hours. The diameter of the zone of inhibition was measured after incubation.

## **RESULTS**

Tables 1-3 shows the results of the antibacterial activity of the crude extracts of the leaf, stem bark and root of *Parkia biglobosa* against MRSA isolates from the wound, skin and beds of orthopaedic patients. The results show that the leaf was not active against the wound isolates at all the concentrations used but showed little activity against the skin and bed isolates at 25mg/ml. Comparatively, the stem bark showed the best activity, followed by the roots and then the leaf. The activities were also found to be concentration dependent.

Table 1

**Antibacterial activities of crude extract of the leaf of *Parkia biglobosa* against MRSA isolates****Diameter of zone inhibition (% of isolates)**

Extract	No Zone			8-10mm			11-14mm		
	wound	skin	bed	wound	skin	bed	wound	skin	bed
10mg/ml.	33(100)	36(100)	48(100)	NZ	NZ	NZ	NZ	NZ	NZ
15mg/ml.	33(100)	36(100)	48(100)	NZ	NZ	NZ	NZ	NZ	NZ
20mg/ml.	33(100)	36(100)	48(100)	NZ	NZ	NZ	NZ	NZ	NZ
25mg/ml.	33(100)	27(75)	31(64.6)	NZN	9(25)	17(35.4)	NZ	NZ	NZ

Key: NZ: no zone, Diameter of cork borer =6mm

Table 3

**Antibacterial activities of crude extract of the stem bark of *Parkia biglobosa* against MRSA isolates****Diameter of zone inhibition (% of isolates)**

Extract	No Zone			8-10mm			11-14mm		
	wound	skin	bed	wound	skin	bed	wound	skin	bed
10mg/ml.	30(90.9)	26(72.2)	28(58.3)	3(9.1)	10(27.8)	20(41.7)	NZ	NZ	NZ
15mg/ml.	26(78.8)	22(61.1)	21(43.8)	7(21.2)	12(33.3)	20(41.7)	NZ	2(5.6)	7(14.6)
20mg/ml.	22(66.7)	6(25.0)	14(29.2)	5(15.2)	14(28.9)	13(27.1)	6(18.2)	13(36.1)	21(43.8)
25mg/ml.	21(63.6)	9(25.0)	11(22.9)	5(15.2)	11(30.6)	9(18.8)	7(21.2)	16(44.4)	28(58.3)

Key: NZ: No zone, Diameter of cork borer =6mm

Table 2

**Antibacterial activities of crude extract of the root of *Parkia biglobosa* against MRSA isolates**

Extract	Diameter of zone inhibition (% of isolates)								
	No Zone			8-10mm			11-14mm		
	wound	skin	bed	wound	skin	bed	wound	skin	bed
10mg/ml.	33(100)	36(100)	48(100)	NZ	NZ	NZ	NZ	NZ	NZ
15mg/ml.	29(87.9)	31(86.1)	36(75.0)	4(12.1)	3(8.3)	9(18.8)	NZ	2(5.6)	3(6.3)
20mg/ml.	26(78.8)	20(55.6)	22(25.8)	6(18.2)	14(38.9)	21(43.8)	1(3.0)	2(5.6)	5(10.4)
25mg/ml.	22(66.7)	14(38.9)	16(33.3)	5(15.2)	15(41.7)	12(25.0)	6(18.2)	7(19.4)	20(41.7)

Key: NZ: no zone, Diameter of cork borer =6mm

## DISCUSSION

It is now a known fact that MRSA has become a threat to both the hospitalized patient and the community globally and that scientists are continuously searching for alternative agents that can be effectively used to control them. This is more with the rate at which bacteria are developing resistance to antibiotics. The discovery that reasonably low concentrations of the crude extracts of parts of *Parkia biglobosa* can be used to inhibit their growth is therefore of tremendous interest. What more, because there has been no known report of *parkia biglobosa* toxicity which is the major problem encountered with most plants.

The extracts of the roots showed no activity against MRSA isolates at 10mg/ml and a very low activity at 15mg/ml. There was however an increased activity at 20mg/ml and 25mg/ml. The crude extracts of the stem bark was active at various concentrations (10mg/ml-25mg/ml) used against MRSA isolates from orthopaedic patients and these activities were concentration dependent. Results obtained

showed that the leaf extract was less active than the other extracts. This agrees with an earlier report of Millogo-Kone et al (2006) when he compared the effects of the extracts of the leaf and stem bark of the same plant against clinical isolates of *Staphylococcus aureus*. Purer extracts of the parts of this plant (Bioactive components) are most likely to achieve better results even at very low concentrations.

*Parkia biglobosa* has been reported to be rich in tannins, flavonoids and saponins among others which are secondary metabolites known to have antibacterial activities. The combined effects of these secondary metabolites may be responsible for the observed activities<sup>8</sup>.

It has been suggested that the presence of MRSA in orthopaedic patients' wound can cause delay in the healing of the wound and subsequent overstay in the hospital or even possible amputations of the affected area. Even though the Staphylococcal isolates used in this work are not confirmed Mec A positive isolates,

the fact that they are methicillin resistant and yet susceptible to a reasonable concentration of the crude extracts of different parts of this plant (especially the stem bark) is a pointer to the great potentials of this plant which needs to be exploited in the treatment of very resistant strains of *Staphylococcus aureus*.

It is known that the problem of antibiotic resistance has limited the use of most known antibiotics and has made the continued search for new antimicrobial compounds inevitable. It is also known that plants produce compounds that can be

effective antimicrobials and resistance modifying agents e.g extracts of the stem bark of *Parkia biglobosa* has been variously reported to be very effective in the control of Staphylococcal infections<sup>2,8,5,6</sup>. The results obtained in this work confirms this and goes further to show that the stem bark of *Parkia biglobosa* has greater antistaphylococcal potentials even against strains of the highly resistant methicillin resistant *Staphylococcus aureus*(MRSA). *Parkia biglobosa* therefore, stands out as a veritable source of new compounds which will serve good purposes in the future.

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