

Original Research Article

ANTIMICROBIAL EFFECTS OF THE STEM BARK EXTRACTS OF *Parkia biglobosa* (Jacq.) G.Don ON *Escherichia coli* AND *Staphylococcus aureus*

ABSTRACT

Aims: This work aimed to evaluate the antimicrobial potential of the stem bark extracts of *P. biglobosa* on selected bacteria species, *Escherichia coli*, and *Staphylococcus aureus*.

Place and Duration of Study: Microbiology laboratory, Department of Biological Sciences, Bingham University-Karu, Nasarawa State, Nigeria.

Methodology: Plant extracts were made by boiling, soaking and methanol extraction, and the antimicrobial assay was performed using the disc and agar-well diffusion methods.

Results. The results indicated all the extract exhibited antimicrobial properties. The highest potential activity was observed with the macerated extract with zones of inhibition at 12.5 mm and 6.5 mm against *S.aureus* and *E.coli*, respectively in the agar-well diffusion method while no active zone of inhibition was recorded in the disk diffusion method. It was noted that the macerated extract showed the highest MIC of 100 mg/ml with a zone of inhibition at 15.0 mm against *S. aureus*.

Conclusion: The results obtained showed that the macerated extract of the plant possess the highest antimicrobial effect against *S. aureus*, and *E. coli*. Although observed potency of this extract *in vitro*, may not be translated to the same activity *in vivo*. However, the results confirmed the efficacy of the plant as a natural antimicrobial and can be further investigated for possible antimicrobial drug discovery.

1.0 INTRODUCTION

1.1 *Parkia biglobosa*

Parkia biglobosa also known as the African locust bean tree, from the family Leguminosae, is a perennial legume and multipurpose tree that is native to West and Central African countries such as Senegal, Gambia, Guinea Bissau, Guinea, Sierra Leone, Mali, Côte d'Ivoire, Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria, Cameroon, Chad, Central African Republic, Democratic Republic of Congo, Sudan and Uganda⁴. The tree currently exists within a wide range of natural communities, including tropical rainforests and arid zones, but is most abundant where cultivation is semi-permanent. It is also present in Australia, South-East Asia, North America and, tropical South America as an introduced species. Several benefits of *P. biglobosa* tree have been reported and these include the vital economic role it plays in recycling nutrients from the soil, its importance as a good source of timber and a valuable food source. The seeds are used as a condiment in food preparation; the husks and pods are good to feed livestock, the

floury pulp, which contains macronutrients, vitamins A and C, can be made into a refreshing drink, and the bark is also used with lemon for wounds and ulcers treatment ⁵.

The use of this plant is quite numerous, especially its Medicinal Uses and, are being used across all tropical countries to cure different ailments. Virtually, all parts of Parkia plants are utilized traditionally for different medicinal purposes. Different parts of Parkia plants are processed as paste, decoction, tincture, powder and juice for the treatment of various ailments (ref). The bark for example is used for cardio protection.⁶ It has been reported that Parkia species are used in different forms to cure diarrhea and dysentery (ref). Different parts of P. biglobosa, and other parkia species have been reported used traditionally, for the treatment of diabetes⁶. Skin-related diseases, such as eczema, skin ulcers, measles, leprosy, wound, dermatitis, chickenpox, scabies, and ringworm have been reported treated using leaves, pods, and roots of other parkia species such as P. speciosa and P. timoriana⁷. The stem barks as well as root of P. biglobosa and other parkia species have been applied in the form of paste and decoction to treat different skin problems⁷. Decoction and paste of stem bark, pod, or root of P. biglobosa have been used to treat hypertension⁸. In addition, stem barks and leaves of P. biglobosa and Parkia species have been used for severe cough and bronchitis treatment⁸. These fore mentioned uses suggested that Parkia plants are likely to contain constituents with broad and diverse biological activities, such as antidiabetic, antimicrobial, antihypertensive, and anti-inflammatory⁸.

1.2 Escherichia coli *Escherichia coli* are rod-shaped, Gram-negative organisms that commonly inhabit the large intestine, and are naturally excreted in feces. The urinary tract is the most common site of *E. coli* infection and more than 90% of all uncomplicated urinary tract infections (UTIs) are caused by *E. coli* infection, particularly in women because of the proximity of the urethra to the anus. *E. coli* UTIs are caused by uropathogenic strains of *E. coli* ^{9,10}.

Over the years, increasing antibiotic resistance in *E. coli*, have raised concern, with some cases of *E. coli* bacteremia reported to be resistant to ciprofloxacin and/or gentamicin. There has been very little change in resistance to these drugs since 2009 and resistance to ciprofloxacin and gentamicin in 2013 remains very similar to 2012¹¹. Resistance to ciprofloxacin decreased early in 2009–13, but has since levelled. Resistance to the third-generation cephalosporin (ceftazidime and cefotaxime) remains stable at 10-11% ¹¹. Several factors have been shown to contribute to the increased risk of antibiotic resistance, including: being a care home resident, recurrent UTI, hospital isolation >7 days in the last 6 months, and unresolved- urinary symptoms. ¹²

1.3 Staphylococcus aureus

Staphylococcus aureus is a, gram-positive non-motile, non-spore forming facultative anaerobe that is biochemically catalase and coagulase positive. It occurs as an irregularly grape-like cluster and sometimes singly or in pairs, typical colonies are smooth raised yellow to golden yellow color and hemolytic on blood agar containing 5% sheep or horse blood¹³. To date, there

are about 40 Staphylococcal species that have been reported, nine of them have two subspecies while one has three subspecies¹⁴. The classification of Staphylococci is not complete yet; new species undergoing validation are still being reported. While some members are important to human medicine, others are relevant to veterinary medicine as they are found in animals or food (ref). Biochemically members of the genus are grouped into two; such as coagulase-positive staphylococci and coagulase-negative staphylococci. *S. aureus* is the most important member of coagulase-positive, causing infection in both humans and animals and is considered the most pathogenic member of the genus staphylococci¹³. Other coagulase positive staphylococcus includes *S. intermedius*, *S. hyicus*, *S. pseudintermedius*, *S. lutrae*, and *S. schleiferi*

Antibiotic resistance in *S. aureus* predates the era of antibiotics used in clinical practice. Before the introduction of penicillin, mortality because of invasive *S. aureus* infection was very high. However, penicillin had a significant effect in reducing the rate of mortality because of *S. aureus* infection, not until 1942 that a strain of *S. aureus* resistant to penicillin was identified first in the hospital and then in the community¹⁵. The use of penicillin as a drug of choice in the treatment of *S. aureus* infection was very effective until the mid-1950s when the number of *S. aureus* resistant to penicillin significantly increased leading to a decrease in the therapeutic value of penicillin¹⁵.¹⁶ reported that about 90% of *S. aureus* are penicillin-resistant. Resistance to penicillin was acquired via the acquisition of plasmids coding for beta-lactam resistance, and it's usually mediated by *Blaz* gene which codes beta lactamase enzymes. Beta lactamase are extracellular enzymes synthesized on exposure to beta lactams class of antibiotics, it hydrolyses the beta lactam ring thereby reducing the therapeutic effect of penicillin¹⁷.

The use of medicinal plants such as *P. biglobosa*, constitutes a significant resource employed by communities for their daily healthcare.¹ Therefore, this study is carried out to summarize current knowledge on the medicinal uses of this plant, screened for its phytochemicals and investigate its antimicrobial potentials.

2.0 MATERIALS AND METHODS

2.1 Area of Study

The study was carried out in Bingham university, located in Auta-balefi, Karu, Nasarawa state, about 26km away from the Capital of Nigeria, along Abuja-Keffi Express way¹⁷. It has a tropical climate with two distinct seasons; rainy and dry seasons. The University covers a land mass of 200 square meters and is geographically found at latitude 8⁰50⁰N and longitude 7⁰52⁰ E.

2.2 Collection of Plant and Microorganism Samples

The bark of *P. biglobosa* stem and roots were collected at Bingham University, Karu and the dead corks on the stem bark was carefully removed. The plant sample was identified by Dr. Jerome Inhuman at the Department of Biological Sciences, Bingham University. The bacteria

samples were collected from Vom Christian Hospital Plateau with the aid of a medical laboratory Scientist. The samples were transported to the Microbiology laboratory of Bingham University for sample processing and analysis.

2.3 Preparation of Plant Samles

Freshly collected stem barks were thoroughly washed with tap water and sliced into small pieces. Then and air-dried at room temperature for four weeks in the laboratory. the dried samples were pounded to powder in a mortar. and the big particles were removed using a laboratory sieve¹⁸. Thereafter, the big particles were blended using a sterile electric blender to increase extract concentration. The plant sample was not exposed to sunlight so that the active compounds are not lost¹⁸.

2.4 Extraction of Plant Samples

2.4.1 Boiled extract

placing 10 g of the small pieces of the plant stem bark was placed in a conical flask and 100 ml of distilled water was added. The mixture was placed on hot plate and allowed to boi for 15 to 20 munites, while stirred regularly at at intervals. After, the mixture was filtered using Whatmann No 1 filter paper and the supernatant recovered. The supernatant was concentrated to dryness using a freezedryer. The extract obtained was stored at 5°C in the refrigerator for further use¹⁸.

2.4.2 Soaked extract

10g of the powdered stem bark was added into 100 ml of the distilled water in a container and covered tightly . The mixture was allowed to stand at room temperature for 48 hours, and occasionally stirred. The mixture was then filtered using the Whatmann No 1 filter paper to remove the residue. The filtrate was allowed to settle for 30 minutes at room temperature. The filtrate was concentrtrted to dryness using a freezedryerThe extract obtained was then stored at 5°C in the refrigerator for further use²⁰.

2.4.3 Macerated extract

10 g of the powdered stem bark was added to 20 ml of distilled water in an air-tight maceration jar. The mixture is allowed to stand at room temperature for 72 hours and occasionally stirred. The mixture was then filtered to separate the plant material from the liquid. The filtrate was allowed to sediment and it was decanted thereafter. The filtrate obtained was cocentrated to dryness using a freezedryer. The extract obtained was stored in the refrigerator at 5°C for further use²⁰.

2.5 Qualitative Phytochemical Analysis

The extract of the plant was screened for the presence of various secondary metabolites (phytochemical) such as alkaloids, saponins, tannins, resins, Cardenolides, Phlobatanins, phenols and, flavonoids¹⁸

2.6 Antimicrobial Assay

2.6.1 Media Preparation and Bacterial Inoculum

The media used were Nutrient agar and Mueller-Hinton agar, which were prepared under an aseptic technique according to manufacturers instructions respectively (HiMedia).

The bacterial strains were subcultured in the prepared nutrient agar and the culture was maintained at 37°C for 24 hours (Mohammed *et al.*, 2017). This was followed with the inoculation of the bacteria strain in a prepared muller Hilton Agar. (Obioma *et al.*, 2017).

2.6.2 Determination of Antimicrobial Activity using Disc Diffusion Method

For the disc diffusion assay, 1 ml of each bacterial suspension was evenly spread on a solidified Muller Hilton Agar. Discs (4 mm in diameter) were punched from sheets of Whatman filter paper, sterilized and, impregnated with 20 µl each of 100 mg/ml plant extract and, dried at room temperature for 12 hours. Thereafter, the discs were placed on the surface of inoculated Muller Hilton agar plate and incubated at 37°C for 24-48 hours for the observation of the formation of inhibition zones around the discs²¹.

2.6.3 Determination of Antimicrobial Activity using Agar-well Diffusion Method

In this method, Muller Hilton agar was prepared and inoculated as in the disc diffusion method. Four (4) holes of 6mm in diameter were made on the inoculated Muller Hilton agar plates using a sterile cork borer and the agar discs were removed. Holes were aseptically filled with 20µl of 100mg/ml of plant extract using microliter pipette, and allowed to diffuse at room temperature for 2 hours and thereafter incubated at 37°C for 24-48 hours. Antibacterial activity was assessed by the appearance of an inhibition zone around the hole, without bacterial growth²¹.

2.7 Analysis

The data was analyzed through representation in tables

3.0 RESULT AND DISCUSSION

Evaluation of the antimicrobial activity of *P.a bigblobosa* extracts; boiled, soaked and, macerated was determined initially by the disc and agar-well diffusion method against *S. aureus* and *E.coli obtainedas* pure isolates from Vom Christian hospital Jos. The result showed that all the *P. bigblobosa* extracts used in this study exhibited a varying degree of antimicrobial activity against all the microorganisms tested. Table 1 showed the highest zone of inhibition at 12.5 mm of the microorganisms against the plant extracts in comparison to the control, in this case, Augmentin which was at 25.0 mm

Augmentin showed the highest zone of inhibition at 18.0 mm (Table 1) in comparison to the

extract which showed no zone of inhibition against the microorganisms, using the disc diffusion method.

Although *E.coli* and *S. aureus* was resistant to all the extracts in the disc diffusion method, but showed zones of inhibition in the macerated extracts of the plant, and resistant to the soaked and boiled extract of the plant in the agar well diffusion method (Table 1 and 2).

Table 1: Diameter of zones of inhibition (mm) of *Parkia biglobosa* extracts against *S. aureus* and *E. coli* at 100 mg/ml by agar-well diffusion method.

Bacterial Isolate	Extracts											
	<i>P. biglobosa</i> (Boiled) (mm)			<i>P. biglobosa</i> (Soaked) (mm)			<i>P.a biglobosa</i> (Macerated) (mm)			Control (Augmentin) (mm)		
	Plate 1	Plate 2	Mean Value	Plate 1	Plate 2	Mean value	Plate 1	Plate 2	Mean value	Plate 1	Plate 2	Mean value
<i>Staphylococcus aureus</i>	6.0	-	6.0	-	-	-	15.0	10.0	12.5	30.0	20.0	25.0
<i>Escherichia coli</i>	-	-	-	-	-	-	8.0	5.0	6.5	30.0	25.0	27.5

Table 2; Diameter of zones of inhibition (mm) of *P. biglobosa* extracts against *S. aureus* and *E. coli* at 100 mg/ml by disc diffusion method.

Bacterial Isolate	Extracts											
	<i>P. biglobosa</i> (Boiled) (mm)			<i>P. biglobosa</i> (Soaked) (mm)			<i>P.a biglobosa</i> (Macerated) (mm)			Control (Augmentin) (mm)		
	Plate 1	Plate 2	Mean Value	Plate 1	Plate 2	Mean Value	Plate 1	Plate 2	Mean Value	Plate 1	Plate 2	Mean value
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	18.0	18.0	18.0
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	20.0	18.0	19.0

Minimum Inhibitory Concentration of *P. biglobosa* macerated extract on *S. aureus*

It was observed that the macerated extract of *P. biglobosa* bark was effective when tested. It showed zones of inhibition against the bacterial pathogen *S. aureus* and *E.coli*. The effectiveness of the extracts in the tested bacterial pathogens was determined by measuring the Minimum Inhibitory Concentration (MIC). MIC was performed on both *S. aureus* and *E.coli*, as it showed a zone of inhibition and was sensitive to the plant extract bark in the previous agar-well diffusion method. The MIC of 1g/ml the macerated extract *P.biglobosa*, while the MIC of *S.aureus* was 50 mg/ml and 25 mg/ml against the boiled extract *P. urinaria*. (ref)

Table 3: MIC values of Macerated extract of *P. biglobosa* plant against *S. aureus*

MIC Values (mg/ml)	Zone of Inhibition (mm)	
	E.coli	S. aureus
100 (stock)	8.0	15
50	5.0	10
10	-	-

Phytochemical Analysis of *Parkia biglobosa*

The phytochemical analysis of *P.biglobosa* plant extracts revealed the presences of alkaloids, flavonoids, tannins, saponins, resins and phenol in the tested extracts (Table 4). These phytochemicals are naturally occurring in most plants, and are known to be biologically active and have bactericidal and fungicidal activities, conferring the antibacterial property to the tested plants^{18,21}.

Table 4: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF *Parkia biglobosa*

Phytochemicals	S(Ethanol)	S(Hot water)	N(ethanol)	N(hot water)	Cl(ethanol)	Cl(hot water)	B(ethanol)	B(hot water)	Ca(ethanol)	Ca(hot water)
Alkaloids	+	+	+	-	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	+	-	+	-	+	-	-
Tanins	-	+	+	+	+	+	+	-	-	-
Phenol	-	+	+	+	+	+	+	-	-	+
Phlobatanins	-	-	-	-	-	-	-	-	-	-
Resins	+	+	-	-	+	+	-	+	-	+
Cardenolides	-	-	-	-	-	-	-	-	-	-

The extracts of *P. biglobosa* obtained by boiling, soaking, and maceration, in this research, showed antimicrobial properties against the bacterial pathogens, *S. aureus* and *E. coli* using the

agar well diffusion method as described by ²². The largest zone of inhibition for *S. aureus* was 15 mm as observed with the macerated extract of the plant. The MIC for *S. aureus* was 100 mg/ml with a zone of inhibition at 15 mm and 50 mg/ml with the zone of inhibition at 10 mm for the macerated extract. The soaked and boiled extracts did not affect the microorganism *S. aureus* and *E.coli* in the disc and agar-well diffusion methods. Augmentin, the control used in this study showed high antimicrobial effect against the organisms compared to the *p. biglobosa* extracts. The highest zone of inhibition was 25 mm for both *S. aureus* and *E.coli*.

The plant extracts were all positive for five out of eight of the phytochemical tests conducted. It shows that they were very rich in Alkaloids, Saponins, Flavonoid, Phenol and Tannins and contains traces of Resins thus confirming our results, no cardolines was present. The extracts were however all tested negative for the Cardenolides test. Our antibacterial results showed that the stem bark of *P. biglobosa*, when macerated at a suitable concentration will be able to suppress the growth of the organism such as ***S. aureus*** and *E. coli*. Although, as reported by ^{18,19} extraction of medicinal plant parts through boiling, soaking and maceration for antimicrobial activity investigation using distilled water as solvent has proven to give good results. This has however confirmed in our study, in addition, the ability of the macerated extract produced the best result against the tested microorganisms, may not be unconnected with the fact the most active antimicrobial constituents resides in the macerated extract. More so, the 72 hours duration of extraction could have encouraged total or overall extraction of the phytoconstituents and thus, enhance the potency of the extract.

Conclusion

In this study, the antimicrobial activity of plants was assessed by disc diffusion and agar-well diffusion methods. The result showed most potential antimicrobial effect with the macerated extract of the plant on *S. aureus*, and *E.coli*. Although observed potency of this extract *in vitro*, may not be translated to the same activity *in vivo*. However, the results confirmed the efficacy of the plant as a natural antimicrobial agent and can be further investigated for possible antimicrobial drug discovery.

Recommendations

Based on the results of this research, the following recommendations are made:

1. Instead of crude aqueous extracts, ethanolic extractions of the plant might exhibit better antimicrobial activity.
 2. Other parts of the plant could be studied to evaluate the studied plant extracts as a potential antimicrobial agent.
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