Original Research paper

Antimicrobial Properties of the African Locust Bean (*Parkia biglobosa*) Effluent and its Chaff

Abstract

Aim: The aim of this study is to determine the antimicrobial properties of the *Parkia biglobosa* (Jacq.) R.Br. ex G.Don effluent. The effluents were tested against some pathogenic microorganisms for their antimicrobial properties using the conventional antibiotics as the control.
Study Design: Effluent with and without chaff is to serve as agents used to determine whether it has antimicrobial properties on the clinical and typed isolates.
Place and Duration of Study: This study was carried out between November, 2015 and July, 2016 at the Department of Microbiology Laboratory, Federal University of Technology Akure, Ondo State, Nigeria.
Methodology: Locust beans bought from "Oja Oba" market, Ikare-Akoko, Ondo state were cooked until the coat was soft and the effluent was decanted, cooked again and the effluent with chaffs was also decanted. Both effluents (with and without chaffs) were used against the test and clinical microorganisms using agar well diffusion method. The Minimum inhibitory concentration was carried out using tube dilution method using Mueller Hinton broth
Results: When the pathogenic microorganisms were subjected to antimicrobial tests using the *P. biglobosa* effluents at 100mg/mL; the effluents and effluent without chaffs were able to inhibit *S. aureus* (ATCC 43300), *S. typhi* (ATCC 35240), *E. coli* (ATCC 35218) while *P. aeruginosa* (ATCC 27853) and *K. pneumonia* (ATCC 48891) were resistant to the effluent. The clinical isolates were more resistant to the effluents. *E.coli* (ATCC 35218) had the lowest susceptibility at 6.33 ± 0.58^{b} and *S. pyogenes* (ATCC 29212) had highest susceptibility with 13.00 ± 1.73^{a} zones of inhibition for locust beans effluent. For the clinical isolate, *E. coli* and *S. pyogenes* had the lowest and highest susceptibility at 4.00 ± 0.00^{b} and 12.33 ± 0.58^{a} respectively for the locust beans effluent while *P. aeruginosa* and *K. pneumoniae* isolates were resistant to both effluents.
. **Conclusion:** This study has provided useful information on the antimicrobial activities of the effluents against clinical and typed microorganisms used in this study.

Keyword: *Parkia biglobosa*, Effluents, Antimicrobial, Antibiotics, Bactericidal.

INTRODUCTION

Parkia biglobosa is a deciduous perennial tree of the Fabaceae family [1] It is popularly known as the Africa locust beans or néré, Dadawa (Hausa), Origili (Ibo) and Iru (Yoruba) [2] It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Processing of locust beans fruits to food condiment, involves different unit operations after harvesting; such unit operations include depodding, and removal of the yellowish pulp to produce locust beans seeds. Other processing operations are cleaning, boiling, dehulling, washing, recooking, and then fermentation to produce the food condiment which is used as soup seasoning/spices [3].

Plate 1: Africa Locust Beans Seeds Used for this Study

The quest for solutions to the global problems of antibiotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobial compounds from a variety of sources including medicinal plants [4]. This is probably because the efficacies of these plant products have been confirmed in different disease situations in different parts of the world and that their little or no known side effects have made them succeed where most synthetic or conventional agents have failed. It may also be because scientists have established that crude extracts of some plants and some pure compounds from such plants can potentiate the activity of antibiotics *in-vitro* [5]. In Africa, medicinal preparations from plants have been used over a long period for the treatment of ailments. This is because orthodox medicine is not available in some places due to a wide range of reasons, among which includes that the first line drugs which are cheap and affordable have become ineffective because of resistance. However, these plant preparations are becoming more widely used by people all over the world as they understand the strength in them and the fact that most of them can be used safely without any known side effect which is not the case in drug or pills [6] This plant has been used extensively for medicinal purposes by the Hausa people of Northern Nigeria and other parts of West Africa. A decoction of the stem bark is used as a mouthwash to steam, relieve toothache as well as a bath for fever and tonic for diarrhoea and as an enema [7, 8, 9]. The leaves are also active against bronchitis, pile, cough, amoebiasis, dental carries and conjunctivitis [10]. The aqueous and acetone extract of *P.biglobosa* raw beans have also demonstrated termicidal properties [11]. There is little or no study on the effluents of *P. biglobosa* as a medium of treatment of ailment, for this reason this study is to determine the antimicrobial properties of the locust beans effluents (with or without chaffs).

2. METHODOLOGY

2.1 Sample Source

The locust beans seeds were purchased at Oja Oba, (King's market) Ikare Akoko, Ondo state, Nigeria and washed thoroughly, they were cooked until the coats of the seeds were soft enough to peel. The effluent (water containing seed coats chaff) was decanted and kept in an air tight container; the peeled seeds were re-washed and re-cooked until it was very soft. Then the effluent (water without chaff) was also decanted and kept in separate air tight container. The samples which were the two different fluents were transported to Microbiology Laboratory of the Federal University of Technology, Akure, Nigeria for additional analyses.

2.2 Source and Preservation of Bacterial Isolates Used

Pure clinical isolates (*E. coli*, *S. typhi*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *S. pneumoniae*) were obtained from the stock culture of States Specialists Hospital, Akure, Ondo State, Nigeria and typed isolates (*E.coli:* ATCC 35218, *S. typhi*: ATCC 35240, *P. aeruginosa*: ATCC 27853, *Kl.pneumoniae*: ATCC 48891, *S. aureus*: ATCC 43300, *S. pneumoniae*) were obtained from Pathological and Clinical Laboratory of Lagos State University Teaching Hospital (Pathcare), Lagos State, Nigeria. Pure isolates were maintained on Nutrient agar slants in the refrigerator at 4^{o}C until further investigative procedure.

2.3 Antibiotic Sensitivity Profile

The antibiotic sensitivity profile was investigated in order to compare the sensitivity of the microorganisms to the different conventional antibiotics. The disc diffusion method was used to determine the susceptibility and resistance of the microorganisms to the antimicrobial drugs. Twenty milliliter of sterile Mueller-Hilton agar was aseptically poured into sterile Petri dishes and allowed to gel. Each plate was seeded with the test organism before aseptically introducing the antibiotic disc with sterile forceps onto the surface of the solidified Mueller Hilton agar plate and incubated at 37^{o}C for 24 hours. After incubation, clear zones around the disk were measured in millimeter and recorded as the zones of inhibition. Diameters of zone of inhibition was measured with a calibrated ruler and then compared with clinical and laboratory standards for their sensitivity or resistance. Seeded plates without antibiotic disk served as the control. The antibiotic sensitivity profile was carried out in triplicates.

2.4 Standardization of Test Microorganism

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours. 0.2 mL was pipetted from the 24 hours broth culture of the test organism and was dispensed into 20 ml sterile nutrient broth and incubated for another 4 hours to standardize the culture to 0.5 McFarland's standard (10^6cfu/mL) before use as described by [12].

2.5 Reconstitution of *P.biglobosa* Effluent

The *P. biglobosa* effluent was filtered with 0.2 µm pore filter membrane and 1mL of the *P.biglobosa* effluent were decanted in 10 mL of Dimethyl Sulfoxide and the concentrate was subjected to antimicrobial activities.

2.6 Determination of Antimicrobial activities of *P. biglobosa* Effluents

Parkia biglobosa (100mg/mL) effluent and the effluent with chaffs were used against the test microorganisms using agar well diffusion method with Chloramphenicol as antibiotic control. Mueller-Hinton and Sabouraud dextrose agar plates were used for bacterial and fungal isolates respectively. Observation and determination of zones of inhibition (ZI) were preceded with an aerobic overnight incubation at 37°C for 24 hours and at 27°C for 48 hours for bacteria and fungi respectively.

2.7 Minimum Inhibitory Concentration (MIC)

The Minimum inhibitory concentration was carried out using tube dilution method using Mueller Hinton broth. The tube dilution susceptibility test was used to determine the MIC values for the locust beans effluent, a series of Mueller-Hinton broth tubes containing varying two fold concentrations of the various *P. biglobosa* effluent samples in the range of 6.25mg/mL to 100mg/mL was prepared and incubated with a previously standardized density of the test microorganisms (0.5mL). The lowest concentration of the *P. biglobosa* effluent samples resulting in no growth following visual inspection after 18-24 hours of incubation for bacteria and 24-72 hours for yeast and mould using spectrophotometer was recorded as the MIC.

2.8 Statistical Analysis

Data obtained were subjected to one way analysis of variance while the mean were compared by Duncan's New Multiple Range Test at 95% confidence interval using Statistical Package for Social Sciences version 16.0. Differences were considered significant at $p \leq 0.05$. Errors were calculated as standard error.

3.0 RESULTS

The Antibiotics sensitivity pattern of the clinical bacteria used for the antimicrobial test against the effluents are shown in Table 1. The clinical bacteria; *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *E. coli* and *S. pyogenes* were tested against some conventional antibiotics using an antibiotics sensitivity disc. The antibiotics used were Septrin (30µg), Ciprofloxacin (10µg), Amoxicillin (10µg), Gentamycin (10µg), Pefloxacin (30µg), Streptomycin (30µg), Ampiclox (30µg), Zinnacef (20µg), Rocephin (25µg), Erythromycin (10µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Augmentin (30µg), and Tarivid (10µg).

The results show that *S. aureus* was susceptible to Septrin, Amoxicillin, Ampiclox, Zinnacef, Rocephin , Chloramphenicol, and Tarivid, with Septrin and Zinnacef having the lowest and highest zones of inhibition at 4.33 ± 0.58^b and 12.67 ± 0.58^b respectively, and it was resistant to the remaining antibiotics; *P. aeruginosa* was inhibited by Rocephin and Tarivid at 4.33 ± 0.58^b and 6.67 ± 0.58^b respectively, which were the lowest and highest zones of inhibition and it was resistant to other antibiotics; *K. pneumonia* was susceptible to Chloramphenicol, Gentamycin, and Tarivid. Gentamycin and Chloramphenicol had the lowest and highest zones of inhibition with 6.00 ± 0.00^a and 8.67 ± 0.58^c respectively.

Table 1: Different Antibiotics concentrations sensitivity pattern of the clinical bacteria

Antibiotics	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Escherichia coli*	*Streptococcus pyogenes*
SXT	4.33±0.58[b]	None	None	None	None	16.00±0.00[e]
CPX	None	None	None	10.33±0.58[c]	14.33±0.58[d]	None
AM	8.33±0.58[c]	None	None	None	10.00±0.00[c]	None
CN	None	None	6.00±0.00[b]	4.33±0.58[b]	10.33±0.58[c]	10.33±0.58[c]
PEF	None	None	None	None	None	4.67±0.58[b]
S	None	None	None	None	None	None
APX	13.00±1.00[d]	None	None	None	4.33±0.58[b]	10.33±0.58[c]
Z	13.33±0.58[d]	None	None	None	None	None
R	7.67±1.15[c]	4.33±0.58[b]	None	None	10.00±0.00[c]	None
E	None	None	None	None	None	14.33±0.58[d]
CH	8.33±0.58[c]	None	8.67±0.58[c]	None	None	None
SP	None	None	None	None	20.33±0.58[e]	None
AU	None	None	None	None	None	None
OFX	12.67±0.58[d]	6.67±0.58[c]	8.33±0.58[c]	12.33±0.58[d]	14.33±0.58[d]	10.67±0.58[c]

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P=0.05).

Legend:

SXT = Septrin (30µg), CPX = Ciprofloxacin (10µg), AM = Amoxicillin (10µg), CN = Gentamycin (10µg), PEF = Pefloxacin (30µg), S = Streptomycin (30µg), APX = Ampiclox (30µg), Z = Zinnacef (20µg), R = Rocephin (25µg), E = Erythromycin (10µg), CH = Chloramphenicol (30µg), SP = Sparfloxacin (10µg), AU = Augmentin (30µg), OFX = Tarivid (10µg)

The antibiotics sensitivity pattern of the typed bacteria used for the antimicrobial test against the effluents is shown in Table 2. The typed bacteria; *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *E.coli* and *S. pyogenes* show almost the same susceptibility to the sensitivity disc as clinical bacteria.

Table 2: **Different Antibiotics concentrations sensitivity pattern of the typed bacteria**

Antibiotics	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Salmonella typhi*	*Escherichia coli*	*Streptococcus pyogenes*
SXT	4.33±0.58[b]	None	None	None	None	16.00±0.00[e]
CPX	None	None	None	10.33±0.58[c]	14.33±0.58[d]	None
AM	8.33±0.58[C]	None	None	None	10.00±0.00[c]	None
CN	None	None	6.00±0.00[b]	4.33±0.58[b]	10.33±0.58[c]	10.33±0.58[c]
PEF	None	None	None	None	None	4.67±0.58[b]
S	None	None	None	None	None	None
APX	13.00±1.00[d]	None	None	None	4.33±0.58[b]	10.33±0.58[c]
Z	13.33±0.58[d]	None	None	None	None	None
R	7.67±1.15[c]	4.33±0.58[b]	None	None	10.00±0.00[c]	None
E	None	None	None	None	None	14.33±0.58[d]
CH	8.33±0.58[c]	None	8.67±0.58[c]	None	None	None
S P	None	None	None	None	20.33±0.58[e]	None
AU	None	None	None	None	None	None
OFX	12.67±0.58[d]	6.67±0.58[c]	8.33±0.58[c]	12.33±0.58[d]	14.33±0.58[d]	10.67±0.58[c]

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P=0.05).

Legend
SXT = Septrin (30µg), CPX = Ciprofloxacin (10µg), AM = Amoxicillin (10µg), CN = Gentamycin (10µg), PEF = Pefloxacin (30µg), S = Streptomycin (30µg), APX = Ampiclox (30µg), Z = Zinnacef (20µg), R = Rocephin (25µg), E = Erythromycin (10µg), CH = Chloramphenicol (30µg), SP = Sparfloxacin (10µg), AU = Augmentin (30µg), OFX = Tarivid (10µg).

Antimicrobial activities of locust beans effluent and effluent with chaffs, on typed microorganisms at 100mg/ml are presented in Table 3. For the typed isolates, at 100mg/ml the effluent of the *P. biglobosa* effluent and with chaffs were able to inhibit *S. aureus* (ATCC 43300), *S. typhi* (ATCC 35240), *E. coli* (ATCC 35218) and *S. pyogenes* (ATCC 29212) while *P. aeruginosa* (ATCC 27853) and *K. pneumoniae* (ATCC 48891) were resistant to the effluent. *E.coli* (ATCC 35218) had the lowest susceptibility at 6.33 ± 0.58^{b} and *S. pyogenes* (ATCC 29212) had highest susceptibility with 13.00 ± 1.73^{a} zones of inhibition for effluent. While for effluent with chaffs *E. coli* (ATCC 35218) and *S. pyogenes* (ATCC 29212) had the lowest and highest zones of inhibition at 4.33 ± 0.58^{a} and 11.33 ± 0.58^{a} respectively while *P. aeruginosa* (ATCC 27853) and *S. pyogenes* (ATCC 29212) had the lowest and highest zones of inhibition at 18.67 ± 0.58^{b} and 24.33 ± 0.58^{c} respectively when tested against Chloramphenicol (control).

Table 3: **Antimicrobial activities of locust beans effluent and with chaffs on typed microorganisms at 100mg/mL**

Microorganisms	ELB	ELBC	C
Staphylococcus aureus (ATCC 43300)	8.67±0.58^{b}	6.00±0.00^{a}	20.33±0.58^{c}
Pseudomonas aeruginosa (ATCC 27853)	None	None	18.67±0.58^{b}
Klebsiella pneumoniae (ATCC 48891)	None	None	19.33±0.58^{b}
Salmonella typhi(ATCC 35240)	12.67±0.58^{b}	9.67±1.15^{a}	24.00±0.00^{c}
Escherichia coli (ATCC 35218)	6.33±0.58^{b}	4.33±0.58^{a}	21.33±0.58^{c}
Streptococcus pyogenes (ATCC 29212)	13.00±1.73^{a}	11.33±0.58^{a}	24.33±0.58^{c}

Data are presented as Mean ± S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P=0.05).
Legend:
ELB- Effluent of locust beans,
ELBC- Effluent of locust beans with chaffs,
C- Chloramphenicol.

Antimicrobial activities of locust beans effluent and with chaffs on clinical bacteria at 100mg/mL are presented in Table 4. For the clinical isolates, *P. biglobosa* effluent with chaffs and without chaffs at 100mg/mL inhibited *S. aureus*, *Salmonella typhi, E. coli*, and *S. pyogenes* while *P. aeruginosa* and *K. pneumonia* were resistant to both effluents. *E. coli* and *S. pyogenes* had the lowest and highest susceptibility at 4.00 ± 0.00^{b} and 12.33 ± 0.58^{a} respectively for the effluent; while *Staphylococcus aureus*, and *S. pyogenes* had the lowest and highest susceptibility at 2.33 ± 0.58^{a} and 9.33 ± 0.58^{a} respectively for effluent with chaffs while *P. aeruginosa* and *K. pneumoniae* isolates were resistant to both effluents. *S. aureus* and *S. pyogenes* had the lowest and highest susceptibility at 16.67 ± 0.58^{c} and 21.33 ± 0.58^{c} respectively when tested against Chloramphenicol (control).

Table 4: Antimicrobial activities of locust beans effluent and effluent with chaffs on clinical microorganisms at 100mg/mL

Microorganisms	ELB	ELBC	C
Staphylococcus aureus	4.00±0.00[b]	2.33±0.58[a]	16.67±0.58[c]
Pseudomonas aeruginosa	None	None	17.33±0.58[b]
Klebsiella pneumonia	None	None	18.67±0.58[b]
Salmonella typhi	10.67±.1.15[b]	8.33±0.58[a]	20.33±0.58[c]
Escherichia coli	4.00±0.00[b]	2.33±0.58[a]	18.67±0.58[c]
Streptococcus pyogenes	12.33±0.58[b]	9.33±0.58[a]	21.33±0.58[c]

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same row are not significantly different (P=0.05).

Legend:

ELB- Effluent of locust beans

ELBC- Effluent of locust beans with chaffs

C- Chloramphenicol.

Minimum inhibitory concentration of *P. biglobosa* effluent and without chaffs in mg/mLis shown in Table 5. When effluents were tested against the typed and clinical isolates, the result showed the MIC of *S. aureus* and *E. coli* (Clinical) was 100mg/mL for both effluents. The MIC of *S. typhi* (ATCC 35240) (Typed) was 50mg/mL for both effluents. The MIC of *S. aureus* (ATCC 43300) (Typed) was 25mg/mL for effluent and 50mg/mL for effluent with chaffs. *P. aeruginosa*, *K. pneumoniae* (Clinical and typed cultured) and *S. typhi* (Clinical) showed no zone of inhibition.

Table 5: Minimum inhibitory concentration of *P. Biglobosa* effluent and effluent with chaffs in mg/mL

Microorganisms	ELB	ELBC
Staphylococcus aureus	100	100
Staphylococcus aureus (ATCC 43300)	25	50
Pseudomonas aeruginosa	NI	NI
Pseudomonas aeruginosa (ATCC 2853)	NI	NI
Klebsiella pneumoniaee	NI	NI
Klebsiella pneumoniae (ATCC 48891)	NI	NI
Salmonella typhi	50	25
Salmonella typhi (ATCC 35240)	50	50
Escherichia coli	100	100
Escherichia coli (ATCC 35218)	50	100
Streptococcus pyogenes	100	50
Streptococcus pyogenes (ATCC 29212)	100	50

Legend:

ELB: Effluent of locust beans,

ELBC: Effluent of locust beans with chaffs.

DISCUSSION

It was discovered that most microorganisms obtained in this study were susceptible to these effluents which implies that the effluents can be used in the treatment of the diseases caused by those microorganisms. The antimicrobial activities of the *P. biglobosa* effluent and effluent with chaffs were tested against clinical and typed microorganisms. The effluent had higher zone of inhibition on the test microorganisms than the effluent with chaffs; this might be as a result of the phytochemical component present in the effluents which could be detrimental to the isolates.The presence of tannins in *P. biglobosa* was confirmed by [7] after studying the phytochemical and antibacterial properties of *P. biglobosa* and its

leaf extracts.The clinical isolates were more resistant compared with the typed microorganisms to the *P. biglobosa* effluents, though the effluent were able to inhibit *S. aureus*, *S. typhi* and *E. coli*. [13] reported that phytochemical screening of the root bark of the plant contains a lot of glycosides and tannins, appreciable amounts of saponins and traces of alkaloids. The presence of such bioactive compounds has been linked to the antibacterial activity such as inhibition of growth [14] and offering some protection to the plant against microbial infections [15]. This findings also correlate with the report of [16] who reported that *P. biglobosa* has been reported to be rich in tannins, flavonoids and saponins among others which are secondary metabolites known to have antibacterial activities.

For both the typed and clinical isolates at 100mg/ml, *P.aeruginosa* (ATCC 27853) and *K. pneumoniae* (ATCC 48891) were resistant the effluents of the *P. biglobosa*. This correlates with the report of [13] who reported that *P. aeruginosa* is however less susceptible, for example bacteria like *P. aeruginosa* has intrinsic antimicrobial resistance due to the permeability of the membrane and has a wide range of efflux pumps. Some strains of *P. aeruginosa* show mutations in the fluoroquinolone binding site, loss of porin channels, and increased production of beta - lactamase as well as cephalosporinase. It may acquire additional resistance mechanisms through external plasmids and has a high potential to be resistant against antimicrobials used during the treatment [7] also reported that *B. cereus* was more susceptible while *P. aeruginosa* was not susceptible to *P. biglobosa* (Jacq.) extract.

CONCLUSION

This study has provided useful information on the antibacterial activity of the effluents against both the typed and clinical microorganisms used in this study. Further work can be carried out on the effluent such as determining the toxic dose and extraction of the bioactive component for use in production of drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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