

## Original Research paper

### Antimicrobial Properties of the African Locust Bean (*Parkia biglobosa*) Effluents with the chaff and without its chaff.

#### Abstract

**Aim:** The aim of this study is to determine the antimicrobial properties of the *Parkia biglobosa* 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 effluents at 100mg/ml; the effluents were able to inhibit *Staphylococcus aureus* (ATCC 43300), *Salmonella typhi* (ATCC 35240), *Escherichia coli* (ATCC 35218) while *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (ATCC 48891) were resistant to the effluent. The clinical isolates were more resistant to the effluents. However, the effluents were able to inhibit *Staphylococcus aureus*, *Salmonella typhi*, *Streptococcus pyogenes* and *Escherichia coli*, while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant to the effluent at 100mg/ml. The effluent without chaffs was found to possess more bactericidal effect on the test microorganisms when compared with the effluent containing chaffs. The highest antibacterial activity of the locust beans effluent without chaffs was recorded for *Salmonella typhi* with 18.00±0.00 inhibition zones and 14.00±0.00 with chaffs respectively. The lowest activity was observed on *Pseudomonas aeruginosa* with the zones of inhibition ranging from 9.67±0.58 and 7.67±0.58 respectively. However, there was no antibacterial activity observed on *Staphylococcus aureus* and *Klebsiella pneumoniae*. **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, re-cooking, and then fermentation to produce the food condiment which is used as soup seasoning/spices [3].



43

44 Plate 1: Africa Locust Beans Seeds Used for this Study

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

66

## 67 2. METHODOLOGY

### 68 2.1 Sample Source

69 The locust beans seeds were purchased at Oja Oba, (King's market) Ikare Akoko, Ondo state, Nigeria  
70 and washed thoroughly, they were cooked until the coats were soft enough to be removed with hands.  
71 The effluent (with chaff) was decanted and kept in an air tight container, while the coats (chaffs) of the  
72 cooked seeds were removed; re-washed and re-cooked until it was very soft. Then the effluent (without  
73 chaff) was also decanted and kept in separate air tight container. The samples were transported to  
74 Microbiology Laboratory of the Federal University of Technology, Akure, Nigeria for additional analyses.

75

### 76 2.2 Source and Preservation of Bacterial Isolates Used

77 Pure clinical isolates (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella*  
78 *pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*) were obtained from the stock culture  
79 of States Specialists Hospital, Akure, Ondo State, Nigeria and typed isolates (*Escherichia coli*: ATCC  
80 35218, *Salmonella typhi*: ATCC 35240, *Pseudomonas aeruginosa*: ATCC 27853, *Klebsiella pneumoniae*:  
81 ATCC 48891, *Staphylococcus aureus*: ATCC 43300, *Streptococcus pneumoniae*) were obtained from  
82 Pathological and Clinical Laboratory of Lagos State University Teaching Hospital (Pathcare), Lagos State,  
83 Nigeria. Pure isolates were maintained on Nutrient agar slants in the refrigerator at 4°C until further  
84 investigative procedure.

85

### 86 2.3 Antibiotic Sensitivity Profile

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

## 97 98 **2.4 Standardization of Test Microorganism**

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

## 103 104 **2.5 Reconstitution of *Parkia biglobosa* Effluent**

105 The *Parkia biglobosa* effluent was filtered with 0.2 µm pore filter membrane and 1ml of the *Parkia*  
106 *biglobosa* effluent were dissolved in 10 ml of Dimethyl Sulfoxide and the concentrate was subjected to  
107 antimicrobial activities.

## 108 109 **2.6 Determination of Antimicrobial activities of *Parkia biglobosa* Effluent**

110 *Parkia biglobosa* effluent (100mg/ml) without chaffs and *Parkia biglobosa* effluent with chaffs were used  
111 against the test microorganisms using agar well diffusion method. Mueller-Hinton and Sabouraud  
112 dextrose agar plates were used for bacterial and fungal isolates respectively. Observation and  
113 determination of zones of inhibition (ZI) were preceded with an aerobic overnight incubation at 37°C for  
114 24 hours and at 27°C for 48 hours for bacteria and fungi respectively.

## 115 116 **2.7 Minimum Inhibitory Concentration (MIC)**

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

## 125 126 **2.8 Statistical Analysis**

127 Numerical data obtained from this study were subjected to analysis of variance; (ANOVA) and the means  
128 were separated by using New Duncan's Multiple Range Tests in SPSS 16.0 computer-aided programme.

129 Errors were calculated as standard error.

## 130 131 **3.0 RESULTS**

132 The Antibiotics sensitivity pattern of the clinical bacteria used for the antimicrobial test against the  
133 effluents are shown in Table 1. The clinical bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*,  
134 *Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Streptococcus pyogenes* were tested  
135 against some conventional antibiotics using an antibiotics sensitivity disc. The antibiotics used were  
136 Septrin (30ug), Ciprofloxacin (10ug), Amoxicillin (10ug), Gentamycin (10ug), Pefloxacin (30ug),  
137 Streptomycin (30ug), Ampiclox (30ug), Zinnacef (20ug), Rocephin (25ug), Erythromycin (10ug),  
138 Chloramphenicol (30ug), Sparfloxacin (10ug), Augmentin (30ug), and Tarivid (10ug).

139 The results show that *Staphylococcus aureus* was susceptible to Septrin, Amoxicillin, Ampiclox,  
140 Zinnacef, Rocephin, Chloramphenicol, and Tarivid, with Septrin and Zinnacef having the lowest and

141 highest zones of inhibition at  $4.33 \pm 0.58^b$  and  $12.67 \pm 0.58^b$  respectively, and it was resistant to the  
 142 remaining antibiotics; *Pseudomonas aeruginosa* was inhibited by Rocephin and Tarivid at  $4.33 \pm 0.58^b$   
 143 and  $6.67 \pm 0.58^b$  respectively, which were the lowest and highest zones of inhibition and it was resistant to  
 144 other antibiotics; *Klebsiella pneumonia* was susceptible to Chloramphenicol, Gentamycin, and Tarivid.  
 145 Gentamycin and Chloramphenicol had the lowest and highest zones of inhibition with  $6.00 \pm 0.00^a$  and  
 146  $8.67 \pm 0.58^c$  respectively.

147

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

149

Antibiotics	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>
SXT	$4.33 \pm 0.58^b$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$16.00 \pm 0.00^e$
CPX	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$10.33 \pm 0.58^c$	$14.33 \pm 0.58^d$	$0.00 \pm 0.00^a$
AM	$8.33 \pm 0.58^c$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$10.00 \pm 0.00^c$	$0.00 \pm 0.00^a$
CN	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$6.00 \pm 0.00^b$	$4.33 \pm 0.58^b$	$10.33 \pm 0.58^c$	$10.33 \pm 0.58^c$
PEF	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$4.67 \pm 0.58^b$
S	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$
APX	$13.00 \pm 1.00^d$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$4.33 \pm 0.58^b$	$10.33 \pm 0.58^c$
Z	$13.33 \pm 0.58^d$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$
R	$7.67 \pm 1.15^c$	$4.33 \pm 0.58^b$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$10.00 \pm 0.00^c$	$0.00 \pm 0.00^a$
E	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$14.33 \pm 0.58^d$
CH	$8.33 \pm 0.58^c$	$0.00 \pm 0.00^a$	$8.67 \pm 0.58^c$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$
SP	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$20.33 \pm 0.58^e$	$0.00 \pm 0.00^a$
AU	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$
OFX	$12.67 \pm 0.58^d$	$6.67 \pm 0.58^c$	$8.33 \pm 0.58^c$	$12.33 \pm 0.58^d$	$14.33 \pm 0.58^d$	$10.67 \pm 0.58^c$

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

152 **Legend:**

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

157  
 158 The antibiotics sensitivity pattern of the typed bacteria used for the antimicrobial test against the effluent  
 159 is shown in Table 2. The typed bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella*  
 160 *pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Streptococcus pyogenes* show almost the same  
 161 susceptibility to the sensitivity disc as clinical bacteria.

162

163

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

Antibiotics	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>
SXT	$4.33 \pm 0.58^b$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$16.00 \pm 0.00^e$
CPX	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$10.33 \pm 0.58^c$	$14.33 \pm 0.58^d$	$0.00 \pm 0.00^a$
AM	$8.33 \pm 0.58^c$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$10.00 \pm 0.00^c$	$0.00 \pm 0.00^a$
CN	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$6.00 \pm 0.00^b$	$4.33 \pm 0.58^b$	$10.33 \pm 0.58^c$	$10.33 \pm 0.58^c$
PEF	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$4.67 \pm 0.58^b$
S	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$
APX	$13.00 \pm 1.00^d$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$4.33 \pm 0.58^b$	$10.33 \pm 0.58^c$

Z	13.33±0.58 <sup>d</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
R	7.67±1.15 <sup>c</sup>	4.33±0.58 <sup>b</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	10.00±0.00 <sup>c</sup>	0.00±0.00 <sup>a</sup>
E	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	14.33±0.58 <sup>d</sup>
CH	8.33±0.58 <sup>c</sup>	0.00±0.00 <sup>a</sup>	8.67±0.58 <sup>c</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
S P	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	20.33±0.58 <sup>e</sup>	0.00±0.00 <sup>a</sup>
AU	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>
OFX	12.67±0.58 <sup>d</sup>	6.67±0.58 <sup>c</sup>	8.33±0.58 <sup>c</sup>	12.33±0.58 <sup>d</sup>	14.33±0.58 <sup>d</sup>	10.67±0.58 <sup>c</sup>

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

167 Legend:

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

172  
 173 Antimicrobial activities of locust beans effluent with chaffs and without chaffs, on typed microorganisms at  
 174 100mg/ml are presented in Table 3. For the typed isolates, at 100mg/ml the effluent of the *Parkia*  
 175 *biglobosa* effluent with chaffs and without chaffs were able to inhibit *Staphylococcus aureus* (ATCC  
 176 43300), *Salmonella typhi* (ATCC 35240), *Escherichia coli* (ATCC 35218) and *Streptococcus pyogenes*  
 177 (ATCC 29212) while *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 48891)  
 178 were resistant to the effluent. *Escherichia coli* (ATCC 35218) had the lowest susceptibility at 6.33 ± 0.58<sup>b</sup>  
 179 and *Streptococcus pyogenes* (ATCC 29212) had highest susceptibility with 13.00 ± 1.73<sup>a</sup> zones of  
 180 inhibition for effluent without chaffs. While for effluent with chaffs *Escherichia coli* (ATCC 35218) and  
 181 *Streptococcus pyogenes* (ATCC 29212) had the lowest and highest zones of inhibition at 4.33 ± 0.58<sup>a</sup> and  
 182 11.33 ± 0.58<sup>a</sup> respectively while *Pseudomonas aeruginosa* (ATCC 27853) and *Streptococcus pyogenes*  
 183 (ATCC 29212) had the lowest and highest zones of inhibition at 18.67 ± 0.58<sup>b</sup> and 24.33 ± 0.58<sup>c</sup>  
 184 respectively when tested against Chloramphenicol (control).

185  
 186 **Table 3: Antimicrobial activities of locust beans effluent with chaffs and without chaffs**  
 187 **on typed microorganisms at 100mg/ml**

Microorganisms	EFWOS	EFWS	C
<i>Staphylococcus aureus</i> (ATCC 43300)	8.67±0.58 <sup>b</sup>	6.00±0.00 <sup>a</sup>	20.33±0.58 <sup>c</sup>
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	18.67±0.58 <sup>b</sup>
<i>Klebsiella pneumoniae</i> (ATCC 48891)	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	19.33±0.58 <sup>b</sup>
<i>Salmonella typhi</i> (ATCC 35240)	12.67±0.58 <sup>b</sup>	9.67±1.15 <sup>a</sup>	24.00±0.00 <sup>c</sup>
<i>Escherichia coli</i> (ATCC 35218)	6.33±0.58 <sup>b</sup>	4.33±0.58 <sup>a</sup>	21.33±0.58 <sup>c</sup>
<i>Streptococcus pyogenes</i> (ATCC 29212)	13.00±1.73 <sup>a</sup>	11.33±0.58 <sup>a</sup>	24.33±0.58 <sup>c</sup>

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

190 Legend:

191 EFWOS- Effluent without chaffs,

192 EFWS- Effluent with chaffs,

193 C- Chloramphenicol.

194

195 Antimicrobial activities of locust beans **effluent with chaffs and without chaffs**, on clinical bacteria at  
 196 100mg/ml are presented in Table 4. For the clinical isolates, *Parkia biglobosa* effluent with chaffs and  
 197 without chaffs at 100mg/ml inhibited *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and  
 198 *Streptococcus pyogenes* while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant to  
 199 both effluents. *Escherichia coli* and *Streptococcus pyogenes* had the lowest and highest susceptibility at  
 200  $4.00 \pm 0.00^b$  and  $12.33 \pm 0.58^a$  respectively **for the effluent without chaffs**; while *Staphylococcus aureus*,  
 201 and *Streptococcus pyogenes* had the lowest and highest susceptibility at  $2.33 \pm 0.58^a$  and  $9.33 \pm 0.58^a$   
 202 respectively for effluent with chaffs while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates  
 203 were resistant to both effluents. *Staphylococcus aureus* and *Streptococcus pyogenes* had the lowest and  
 204 highest susceptibility at  $16.67 \pm 0.58^c$  and  $21.33 \pm 0.58^c$  respectively when tested against  
 205 Chloramphenicol (control).  
 206

207 **Table 4: Antimicrobial activities of locust beans effluent with chaffs and without chaffs on**  
 208 **clinical microorganisms at 100mg/ml**

Microorganisms	EFWOS	EFWS	C
<i>Staphylococcus aureus</i>	$4.00 \pm 0.00^b$	$2.33 \pm 0.58^a$	$16.67 \pm 0.58^c$
<i>Pseudomonas aeruginosa</i>	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$17.33 \pm 0.58^b$
<i>Klebsiella pneumoniae</i>	$0.00 \pm 0.00^a$	$0.00 \pm 0.00^a$	$18.67 \pm 0.58^b$
<i>Salmonella typhi</i>	$10.67 \pm 1.15^b$	$8.33 \pm 0.58^a$	$20.33 \pm 0.58^c$
<i>Escherichia coli</i>	$4.00 \pm 0.00^b$	$2.33 \pm 0.58^a$	$18.67 \pm 0.58^c$
<i>Streptococcus pyogenes</i>	$12.33 \pm 0.58^b$	$9.33 \pm 0.58^a$	$21.33 \pm 0.58^c$

209 Data are presented as Mean $\pm$ S.D (n=3). Values with the same superscript letter(s) along the same row  
 210 are not significantly different (P $\leq$ 0.05).

211 **Legend:**

212 **EFWOS- Effluent without chaffs,**

213 **EFWS- Effluent with chaffs,**

214 **C- Chloramphenicol.**  
 215

216 Minimum inhibitory concentration of *Parkia biglobosa* effluent without chaffs and effluent with chaffs in  
 217 mg/ml is shown in Table 5. When effluents were tested against the typed and clinical isolates, the result  
 218 showed the MIC of *Staphylococcus aureus* and *Escherichia coli* (Clinical) was 100mg/ml for both  
 219 effluents. The MIC of *Salmonella typhi* (ATCC 35240) (Typed) was 50mg/ml for both effluents. The MIC of  
 220 *Staphylococcus aureus* (ATCC 43300) (Typed) was 25mg/ml for effluent without chaffs and 50mg/ml for  
 221 effluent with chaffs. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Clinical and typed cultured) and  
 222 *Salmonella typhi* (Clinical) showed no zone of inhibition.  
 223

224 **Table 5: Minimum inhibitory concentration of *Parkia Biglobosa* effluent without chaffs**  
 225 **and effluent with chaffs in Mg/MI**

Microorganisms	EFWOS	EFWS
<i>Staphylococcus aureus</i>	100	100
<i>Staphylococcus aureus</i> (ATCC 43300)	25	50
<i>Pseudomonas aeruginosa</i>	NI	NI
<i>Pseudomonas aeruginosa</i> (ATCC 2853)	NI	NI
<i>Klebsiella pneumoniae</i>	NI	NI
<i>Klebsiella pneumoniae</i> (ATCC 48891)	NI	NI
<i>Salmonella typhi</i>	50	25
<i>Salmonella typhi</i> (ATCC 35240)	50	50
<i>Escherichia coli</i>	100	100
<i>Escherichia coli</i> (ATCC 35218)	50	100

<i>Streptococcus pyogenes</i>	100	50
<i>Streptococcus pyogenes</i> (ATCC 29212)	100	50

226 Legend:

227 **EFWOS: Effluent without chaffs,**

228 **EFWS: Effluent with chaffs.**

## 229 DISCUSSION

230 It was discovered that most microorganisms obtained in this study were susceptible to these effluents  
 231 which implies that the effluents can be used in the treatment of the diseases caused by those  
 232 microorganisms. **The antimicrobial activities of the *Parkia biglobosa* effluent with chaffs and without the**  
 233 **chaffs were tested against clinical and typed microorganisms. The effluent without chaffs had higher zone**  
 234 **of inhibition on the test microorganisms than the effluent with chaffs;** this might be as a result of the  
 235 phytochemical component present in the effluents which could be detrimental to the isolates. The  
 236 presence of tannins in *Parkia biglobosa* was confirmed by [7] after studying the phytochemical and  
 237 antibacterial properties of *Parkia biglobosa* and its leaf extracts. The clinical isolates were more resistant  
 238 compared with the typed microorganisms to the *Parkia biglobosa* effluents. Though it was able to inhibit  
 239 *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*, but *Pseudomonas aeruginosa* and  
 240 *Klebsiella pneumoniae* were resistant to the effluents at 100mg/ml, **this may be due to the fact that the**  
 241 **patients from which the clinical isolates were obtained from had probably been introduced to one**  
 242 **antibiotics or the other which is not effective in inhibiting their growth and as a result, have become more**  
 243 **resistant.** Generally, it was discovered that microorganisms obtained in this study, are susceptible to  
 244 these effluents which implies that the effluents can be used in the treatment of the diseases caused by  
 245 those microorganisms. [13] reported that phytochemical screening of the root bark of the plant  
 246 contains a lot of glycosides and tannins, appreciable amounts of saponins and traces of alkaloids.  
 247 The presence of such bioactive compounds has been linked to the antibacterial activity such as  
 248 inhibition of growth [14] and offering some protection to the plant against microbial infections [15].  
 249 This findings also correlate with the report of [16] who reported that *Parkia biglobosa* has been reported  
 250 to be rich in tannins, flavonoids and saponins among others which are secondary metabolites  
 251 known to have antibacterial activities.

252 For the typed isolates at 100mg/ml, the effluents of the *Parkia biglobosa* were resistant to *Pseudomonas*  
 253 *aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 48891). This correlates with the report of  
 254 [13] who reported that *Pseudomonas aeruginosa* is however less susceptible, it was observed that the  
 255 leaves and seeds of *Parkia biglobosa* (Jacq.) were active against *Staphylococcus aureus*, *Bacillus*  
 256 *cereus*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *C. utilis*. [7] also reported that *Bacillus cereus*  
 257 was more susceptible while *Pseudomonas aeruginosa* was not susceptible to *Parkia biglobosa* (Jacq.)  
 258 extract.

259

## 260 CONCLUSION

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

## 264 COMPETING INTERESTS

265 Authors have declared that no competing interests exist.

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