

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 pneumoniae* (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*, Effluent, Antimicrobial, Antibiotics, Bactericidal.

INTRODUCTION

Parkia biglobosa, also known as the African locust beans or néré, is a deciduous perennial tree of the Fabaceae family [1] It is popularly known as the Africa locust beans, 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].



42

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

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

65

66 2. METHODOLOGY

67 2.1 Sample Source

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

74

75 2.2 Source and Preservation of Bacterial Isolates Used

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

84

85 2.3 Antibiotic Sensitivity Profile

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

96 97 **2.4 Standardization of Test Microorganism**

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

102 103 **2.5 Reconstitution of *Parkia biglobosa* Effluent**

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

107 108 **2.6 Determination of Antimicrobial activities of *Parkia biglobosa* Effluent**

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

114 115 **2.7 Minimum Inhibitory Concentration (MIC)**

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

124 125 **2.8 Statistical Analysis**

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

129 130 **3.0 RESULTS**

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

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

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

146

147 **Table 1: Antibiotics sensitivity pattern of the clinical bacteria**

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 ± 0.58^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	16.00 ± 0.00^e
CPX	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	10.33 ± 0.58^c	14.33 ± 0.58^d	0.00 ± 0.00^a
AM	8.33 ± 0.58^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	10.00 ± 0.00^c	0.00 ± 0.00^a
CN	0.00 ± 0.00^a	0.00 ± 0.00^a	6.00 ± 0.00^b	4.33 ± 0.58^b	10.33 ± 0.58^c	10.33 ± 0.58^c
PEF	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	4.67 ± 0.58^b
S	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
APX	13.00 ± 1.00^d	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	4.33 ± 0.58^b	10.33 ± 0.58^c
Z	13.33 ± 0.58^d	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
R	7.67 ± 1.15^c	4.33 ± 0.58^b	0.00 ± 0.00^a	0.00 ± 0.00^a	10.00 ± 0.00^c	0.00 ± 0.00^a
E	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	14.33 ± 0.58^d
CH	8.33 ± 0.58^c	0.00 ± 0.00^a	8.67 ± 0.58^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
SP	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	20.33 ± 0.58^e	0.00 ± 0.00^a
AU	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
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

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

150 Legend:

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

155
 156 The antibiotics sensitivity pattern of the typed bacteria used for the antimicrobial test against the effluent
 157 is shown in Table 2. The typed bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella*
 158 *pneumoniae*, *Salmonella typhi*, *Escherichia coli* and *Streptococcus pyogenes* show almost the same
 159 susceptibility to the sensitivity disc as clinical bacteria.

160

161

162 **Table 2: Antibiotics 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 ± 0.58^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	16.00 ± 0.00^e
CPX	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	10.33 ± 0.58^c	14.33 ± 0.58^d	0.00 ± 0.00^a
AM	8.33 ± 0.58^c	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	10.00 ± 0.00^c	0.00 ± 0.00^a
CN	0.00 ± 0.00^a	0.00 ± 0.00^a	6.00 ± 0.00^b	4.33 ± 0.58^b	10.33 ± 0.58^c	10.33 ± 0.58^c
PEF	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	4.67 ± 0.58^b
S	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
APX	13.00 ± 1.00^d	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	4.33 ± 0.58^b	10.33 ± 0.58^c
Z	13.33 ± 0.58^d	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

R	7.67±1.15 ^c	4.33±0.58 ^b	0.00±0.00 ^a	0.00±0.00 ^a	10.00±0.00 ^c	0.00±0.00 ^a
E	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	14.33±0.58 ^d
CH	8.33±0.58 ^c	0.00±0.00 ^a	8.67±0.58 ^c	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
S P	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	20.33±0.58 ^e	0.00±0.00 ^a
AU	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
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

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

165 Legend:

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

170
 171 Antimicrobial activities of locust beans effluent with chaffs and without chaffs, on typed microorganisms at
 172 100mg/ml are presented in Table 3. For the typed isolates, at 100mg/ml the effluent of the *Parkia*
 173 *biglobosa* effluent with chaffs and without chaffs were able to inhibit *Staphylococcus aureus* (ATCC
 174 43300), *Salmonella typhi* (ATCC 35240), *Escherichia coli* (ATCC 35218) and *Streptococcus pyogenes*
 175 (ATCC 29212) while *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 48891)
 176 were resistant to the effluent. *Escherichia coli* (ATCC 35218) had the lowest susceptibility at 6.33 ± 0.58^b
 177 and *Streptococcus pyogenes* (ATCC 29212) had highest susceptibility with 13.00 ± 1.73^a zones of
 178 inhibition for effluent without chaffs. While for effluent with chaffs *Escherichia coli* (ATCC 35218) and
 179 *Streptococcus pyogenes* (ATCC 29212) had the lowest and highest zones of inhibition at 4.33 ± 0.58^a and
 180 11.33 ± 0.58^a respectively while *Pseudomonas aeruginosa* (ATCC 27853) and *Streptococcus pyogenes*
 181 (ATCC 29212) had the lowest and highest zones of inhibition at 18.67 ± 0.58^b and 24.33 ± 0.58^c
 182 respectively when tested against Chloramphenicol (control).

183
 184 **Table 3: Antimicrobial activities of locust beans effluent with chaffs and without chaffs**
 185 **on typed microorganisms at 100mg/ml**

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

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

188 Legend:

189 EFWOS- Effluent without chaffs,
 190 EFWS- Effluent with chaffs,
 191 C- Chloramphenicol.

192

193 Antimicrobial activities of locust beans effluent with chaffs and without chaffs, on clinical bacteria at
 194 100mg/ml are presented in Table 4. For the clinical isolates, *Parkia biglobosa* effluent with chaffs and
 195 without chaffs at 100mg/ml inhibited *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and
 196 *Streptococcus pyogenes* while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were resistant to
 197 both effluents. *Escherichia coli* and *Streptococcus pyogenes* had the lowest and highest susceptibility at
 198 4.00 ± 0.00^b and 12.33 ± 0.58^a respectively for the effluent without chaffs; while *Staphylococcus aureus*,
 199 and *Streptococcus pyogenes* had the lowest and highest susceptibility at 2.33 ± 0.58^a and 9.33 ± 0.58^a
 200 respectively for effluent with chaffs while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolates
 201 were resistant to both effluents. *Staphylococcus aureus* and *Streptococcus pyogenes* had the lowest and
 202 highest susceptibility at 16.67 ± 0.58^c and 21.33 ± 0.58^c respectively when tested against
 203 Chloramphenicol (control).
 204

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

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

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

209 **Legend:**

210 EFWOS- Effluent without chaffs,
 211 EFWS- Effluent with chaffs,
 212 C- Chloramphenicol.
 213

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

222 **Table 5: Minimum inhibitory concentration of *Parkia Biglobosa* effluent without chaffs**
 223 **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

224 Legend:

225 EFWOS: Effluent without chaffs,

226 EFWS: Effluent with chaffs.

227 DISCUSSION

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

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

257

258 CONCLUSION

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

262 COMPETING INTERESTS

263 Authors have declared that no competing interests exist.

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