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Original Research Article

3 **Phytochemical Analysis and Antimicrobial Susceptibility of Methanol Leaf extract of**
4 ***Prosopis africana*****(Guill. & Perr.) Taub. (African mesquite) against Selected**
5 **Microorganisms**

6

7 **Abstract**

8 **Introduction:** The idea that certain plants had healing potential was known long before
9 human beings discovered the existence of pathogens. **Methodology:** The crude
10 methanolic leaf extract of *Prosopis africana* was assayed for antimicrobial potency using
11 Agar-well diffusion technique against *Salmonella typhi*, *Klebsiella pneumoniae*,
12 *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant
13 *Staphylococcus aureus* (MRSA) and *Candida albicans*. Qualitative phytochemical
14 screening was also carried out. **Results:** The results of the antimicrobial screening
15 showed antimicrobial potency against the test isolates with various degrees of zone of
16 inhibition which varied between 10mm – 22mm. The highest zone was reported against
17 *Klebsiella pneumoniae* (22mm), followed by *Streptococcus pyogenes* and *Candida*
18 *albicans* (21mm), *Salmonella typhi* (20mm), MRSA (19 mm) and then *Pseudomonas*
19 *aeruginosa* (18mm). Chloramphenicol and Fluconazole are used as reference standard
20 and their zones of inhibitions ranged from 26mm–29mm. The Minimum Inhibitory
21 Concentration (MIC) of the extract ranged between 12.50mg/mL – 50.00mg/mL whilst
22 the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration
23 (MFC) of the extract were at 50.00mg/mL. The result of phytochemical screening
24 revealed the presence of carbohydrates, flavonoids, phenols/tannins, saponins,
25 terpenes, steroids, cardiac glycosides and alkaloids as secondary metabolites.
26 **Conclusion:** The findings clearly showed that methanolic leaf extract of *P. africana* has
27 proved its use in folklore as an alternative antimicrobial agent and further research can
28 lead to isolation of a new lead of medical importance.

29 **Keywords**: Antimicrobial Susceptibility, *Prosopis africana*, Phytochemical Constituents,
30 96-well microplates, MIC, MBC, MFC.

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32 **1. Introduction**

33 Infectious diseases are particularly major challenges to public health, despite
34 tremendous scientific discovery of medicines for their treatment [1]. This is due to
35 increase in resistance to antibiotics by pathogenic microorganisms as a result of misuse
36 and over prescription of antibiotics which has affected our ability to treat patients
37 empirically [2,3]. So in recent years, there has been continuous and urgent need to
38 discover new antimicrobial compounds with diverse chemical structures and novel
39 mechanisms of action because the incidence of new and re-emerging infectious
40 diseases and development of resistance to the antibiotics in current clinical use [3,4].
41 However, nature is endowed with providing continuous new biomolecules with novel
42 structures that are developed to interact with biological systems in providing defense
43 against infectious diseases are of paramount important [5].

44 The presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and
45 essential oils amongst others are responsible for the medicinal properties of plants [6]. It
46 is necessary to screen medicinal plants for the presence of these bioactive chemicals
47 which may lead to a new active principle. Scientific studies available on medicinal plants
48 indicate that promising phytochemical can be developed for many health problems [7].
49 The benefits of using plant derived medicines are that they are relatively cheaper and
50 stable.

51 *Prosopis africana* (Guill. & Perr.) Taub. belongs to family fabaceae. It is a renowned
52 and versatile tree of immense economic value amid the rural communities in the Guinea
53 savanna of Nigeria. It is mostly found in savanna countries of Africa like Senegal and
54 Nigeria [8]. Literature gives an account of its uses as folk medicines for several ailment
55 and virtually all its parts are of medicinal value. Abah et al. (2015) [9] reported that the
56 stem bark is used as remedies for dysentery, gonorrhea, bronchitis and skin diseases.
57 In Niger State of Nigeria, the twigs, leaves, bark, and secondary roots are used for
58 treatment and relieve of typhoid fever, dental decay, malaria as well as stomach cramps
59 while, Atawodi et al. (2002) [10] attested that the bark and root decoctions are utilized
60 for the treatment of Trypanosomiasis in cattle and on lesions as a lotion.

61 It is in knowledge domain that antimicrobial resistance is a great challenge to holistic
62 treatment of infectious diseases as a result of either the use of substandard antibiotics,
63 misuse or over prescription [9]. There is need for searching and discovering of new
64 lead principles that will be effective, safe, readily available and cost effective for these
65 challenges would go a long way of solving such challenges. Therefore, this study was
66 aimed in determining the phytochemical and antimicrobial properties of *P. africana*
67 towards the development of new antimicrobial agent.

68 **2.0 Materials and Methods**

69 **2.1 Plant Sample Collection and Identification**

70 The leaves of *P. africana* were collected in Bida, Niger State, Nigeria. The voucher
71 specimen was prepared, the plant was identified and voucher specimen was deposited
72 in herbarium unit of the Department of Medicinal Plant Research and Traditional
73 Medicine (MPR&TM), National Institute for Pharmaceutical Research and Development
74 (NIPRD), Abuja, Nigeria.

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75 **2.2 Preparation and Preservation of Plant Material**

76 The leaves were properly washed under clean-running tap water to remove the dirt and
77 air dried at room temperature for a week. The dried leaves were pulverized into powder
78 with clean wooden pestle and mortar, and sieved. The pulverized sample was stored in
79 a clean plastic container, properly labeled and tightly covered at 37°C prior for further
80 analysis [11].
81
82 **2.3 Extraction of Crude Extract**
83
84 One hundred grams (100g) of the pulverized leave of *P. africana* was accurately
85 weighed and subjected to cold maceration in 500mL of absolute methanol for 72 h at
86 laboratory temperature. The macerated extract was filtered using Whatman No.1 filter
87 paper. The extraction was repeated for the maximum extraction of the active
88 ingredients and to also obtain reasonable yield (crude extract). The filtrate was dried
89 using water bath at 45°C until all the solvent evaporated out [11,12].

90 The percentage yield of the crude extract (PYCE) was calculated using the formula by
91 [11].

$$PYCE = \frac{Mass\ of\ the\ crude\ extract\ obtained}{Mass\ of\ the\ pulverized\ plant\ sample} \times 100$$

92

93

94 **2.4 Phytochemical Screening of the Extract**

95 The qualitative phytochemical screening of the leaves of *P. africana* was carried out in
96 Pharmacognosy unit, Department of Medicinal Plant Research and Traditional
97 Medicine, NIPRID, Idu-Abuja. The standard methods illustrated by [12-14] were
98 adopted to test for the presence of carbohydrates, flavonoids, phenols/tannins,
99 saponins, terpenes, steroids, alkaloids and cardiac glycosides.

100 **2.5 The Test Microorganisms**

101

102 Antimicrobial activity of methanol extract of leaves of *P. africana* was investigated
103 against five bacterial isolates and one fungal isolate which were obtained from Vaccine
104 Discovery and Research Laboratory, Centre for Genetic Engineering and
105 Biotechnology, Federal University of Technology, Minna, Nigeria. The bacteria strains
106 used for the study include *Salmonella typhi, Klebsiella pneumoniae*, *Streptococcus*
107 *pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus*
108 (MRSA) and the fungi used for the study was *Candida albicans*. The tested bacteria
109 were maintained on Nutrient agar at 37°C for 24 h and *Candida albicans* on Potatoes
110 Dextrose Agar at 30°C for 48-72 h.

111 **2.6 Inoculum Preparation**

112 A loopful of isolated colonies were inoculated into 4 mL sterile Mueller-Hinton Broth
113 (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and incubated at

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114 37°C for 2 h. The turbidity of actively growing microbial suspensions were adjusted with
115 freshly prepared MHB and SDB using $BaSO_4$ turbidity standard to match turbidity
116 standard of 0.5 McFarland. This turbidity was equivalent to approximately $1.5x10^8$
117 CFU/mL cells for bacteria, and $1.5x10^7$ spores/mL for the fungi strain. The grown
118 suspension was used for further testing.

119 **2.7 Preparation of Crude Extract**

120

121 For the preparation of the stock solution, 0.5g of the crude extract was accurately
122 weighed using analytical weighing balance into a sterile tube containing 1mL of 2%
123 Dimethylsulfoxide (DMSO). This was vortexed to allow the extract to completely
124 dissolve and 9mL of sterile distilled water was added to give final extract concentration
125 of 50mg/mL. Double fold dilutions was carried out to give extract concentrations of
126 25mg/mL, 12.5mg/mL and 06.25mg/mL using sterile distilled water respectively.

127 **2.8 *In-vitro* Antimicrobial Susceptibility Assay of the Extract**

128

129 Susceptibility test of the extract against the isolates were determined in the Microbiology
130 Laboratory, Ibrahim Badamasi Babangida University Lapai in Niger State, Nigeria using
131 Kirby-Bauer agar diffusion method according to NCCLS standards ([15,16]. The
132 Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were used for the
133 antimicrobial activity test. About 100 µL of MHB and SDB cultures containing 0.5
134 McFarland equivalents to approximately $1.5x10^8$ CFU/mL cells for bacteria, and $1.5x10^7$
135 spores/mL for fungi strain were dispensed into empty sterile Petri dishes using
136 micropipettes. Twenty-three millilitres (23mL) of sterilized MHA and SDA maintained
137 between 50 – 45°C was added to the appropriate Petri dishes and rocked gently for
138 even distribution of the organisms under aseptic condition and allowed to gel under
139 safety hood for 1 h. On each of the plates containing bacteria isolates. Five wells of 8
140 mm in diameter were made on the agar plates using sterile metallic cork borer and
141 labelled properly. The base of the wells was sealed with 30 µL of MHA and SDA.
142 Thereafter, 200µL of different concentrations of the extract were carefully and
143 aseptically added with the aid of micropipette into each well and left in the safety hood
144 for 2 h for proper diffusion of the extracts into the agar and then incubated at 37°C for
145 24 h for bacteria. The same procedure was repeated for fungi strain and incubated at
146 25°C for 48 h for fungi. The experiment was set up in duplicates. The plates were
147 observed for activity and zones of inhibitions were measured and recorded as mean
148 zone of inhibition. The diameter of each zone was accurately measured with a spotless
149 and translucent ruler in millimetre (mm) in vertical and horizontal manner and mean was
150 determined.

151 Control experiments were set up by using standard antibiotics, Chloramphenicol
152 (250mg) for bacteria strain and fluconazole (80mg) for fungi specie as reference
153 standards for positive control. Sterile MHA and SDA plates were used as Media Sterility
154 Control (MSC) and MHA and SDA plates with the used organisms streaked as
155 Organism Viability Control (OVC). All the controls were given the same treatment as the
156 experiments [15,16].

157 **2.9 Determination of Minimum Inhibitory Concentration (MIC)**

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158 The minimum inhibitory concentration (MIC) value of the extract of *P. africana* leaves
159 extract was determined by microdilution broth method in 96-well microplates [17].
160 Chloramphenicol (Fidson, Nigeria) and Fluconazole (Pfizer, UK) were used as the
161 standard drug for bacteria and fungi at stock concentration of 50µg/ml. Controls of
162 sterility for the Mueller-Hinton nutrient broth, control culture (inoculum),
163 Chloramphenicol, Fluconazole, crude extract and DMSO were carried out. The
164 microwell plates were closed and incubated aerobically at 37°C for 24 h. Thereafter,
165 50µL of tetrazolium dye was applied into each well with 2 h further incubation at 37°C
166 and colour change was observed. Any well with reddish-pink colour signifies the
167 microbial growth, which was noted and documented as positive (MIC). All assays were
168 carried out in triplicate.

169 MIC of extracts was carried against the isolates using the broth microdilution method
170 (BMM) in 96 micro wells titre plates using NCCLS method [16] with little modification. A
171 volume of 50µL of the extract was dispensed into first row and the same volume of the
172 sterilized media (MHB and SDB) was dispensed into each well except the first row. A
173 two-fold dilution was carried out from row 2 to 7 by taking 50µL of the extract to the next
174 row, mixed well and the serial dilution continued. At row 7, 50µL of the final mixture was
175 discarded. Then, 50µL of 0.5 McFarland of 2 h culture was added to each well in row 1-
176 7. The rows 8 and 9 were the OVC and MSC. The plates were incubated at 37°C for 24
177 h. The test was carried out in duplicate and the values are express in mean.

178 **2.10 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC**
179 **and MFC)**
180
181 The MBC and MFC were determined from the Minimum Inhibitory Concentration (MIC)
182 result by subculturing from the wells that shows no any sign of turbidity in the MIC test
183 and streak on the freshly prepared MHA and SDA plates and incubate at 37°C for 24 to
184 48 h and the plates were checked for the present or absent of the growth [16,17].

185 **3.0 Results**
186
187 **3.1 Phytochemical Constituents**
188
189 Qualitative biological active compounds of the methanolic crude extract disclosed the
190 occurrence of carbohydrates, flavonoids, phenols/tannins, saponins, terpenes, steroids,
191 cardiac glycosides and alkaloids respectively as demonstrated in table 1 below.

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193 **3.2 Antimicrobial Activity**
194
195 The methanolic crude extract has antimicrobial efficacy against all the test isolates at 50
196 and 25 mg/mL of concentration, while *Salmonella typhi*, *Klebsiella pneumoniae*,
197 *Streptococcus pyogenes* and Methicillin-Resistant *Staphylococcus aureus* (MRSA)
198 showed activity even at 12.50mg/mL. The extract at 0.625mg/mL however had no
199 activity against all the tested organisms. The antimicrobial controls were active against
200 all tested organisms except *Pseudomonas aeruginosa* (Figure 1).

201 **3.3 Minimum Inhibitory and Bactericidal Concentrations of the Crude Extract**
202 **against Sensitive Organisms**
203
204 Minimum Inhibitory Concentration (MIC) was carried out on all the isolates where
205 *Salmonella typhi* and *Streptococcus pyogenes* had MIC of 12.50mg/mL. MRSA,
206 *Klebsiella pneumoniae* and *C. albicans* had MIC of 25mg/mL while the MIC of
207 *Pseudomonas aeruginosa* was at 50mg/mL (Table 2). The MBC of *Salmonella typhi*,
208 *Klebsiella pneumoniae*, *Streptococcus pyogenes*, MRSA were at 50mg/mL and *C.*
209 *albicans* had the MFC of 50mg/mL (Table 3).

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216 **Table1: Phytochemical constituents of methanolic leaves**

Phytochemicals	Tests	Inference
Carbohydrates	Molisch	+
Flavonoids	Alkaline	+
Phenol/Tannins	Ferric Chloride	+
Saponins	Froth	+
Terpenes	Liebermann	+
Steroids	Salkowski	+
Alkaloids	Dragendoff's	+
	Hagner's	+
	Wagner's	+
Cardiac glycosides	Keller-Kilani	+

217 Key: + = Present - = Absent

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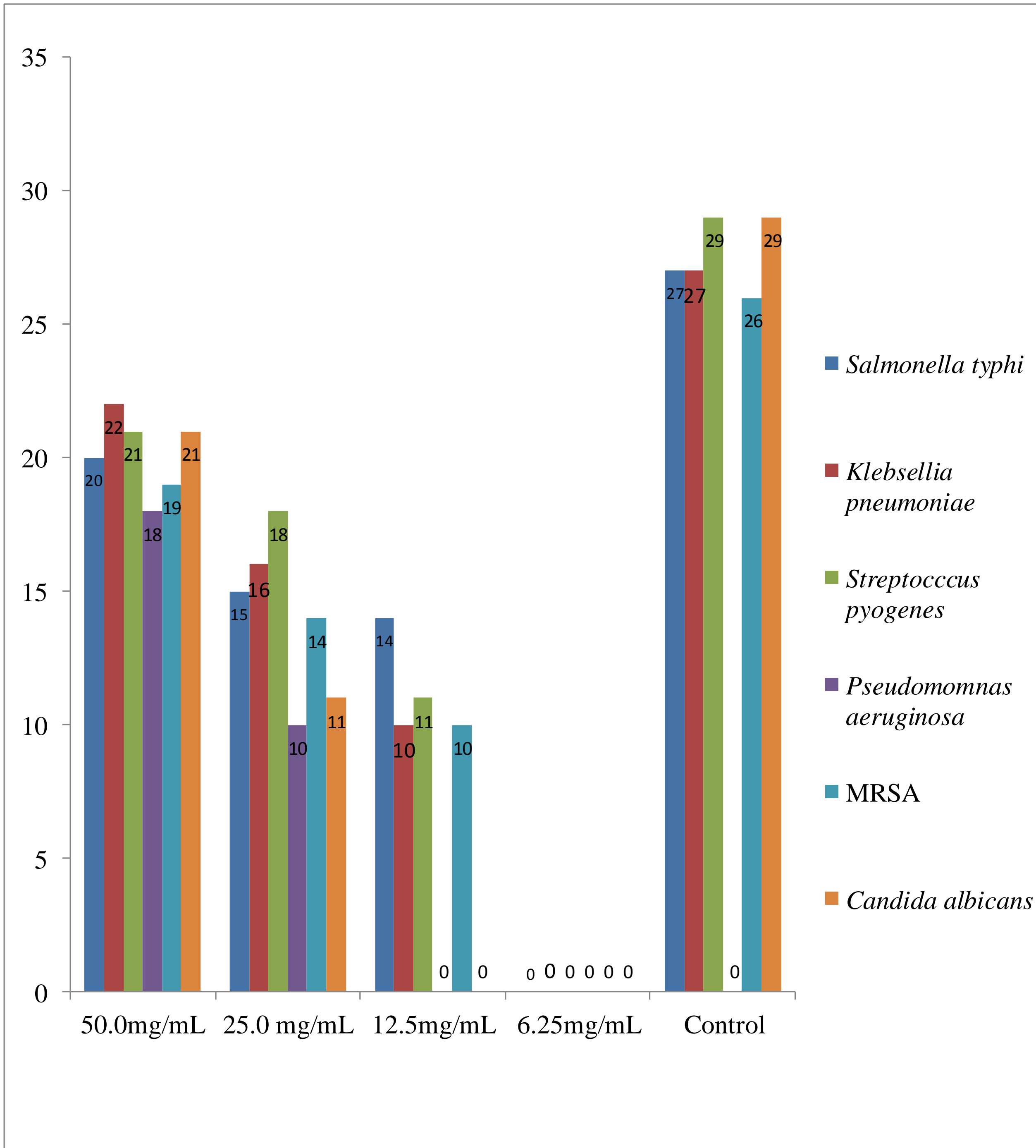

218 **Fig. 1**: Mean zone of inhibition(mm) of methanolic extract of *P. africana* on the test
219 organisms
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224 **Table 2:** Minimum Inhibitory Concentration of Methanol Extract of *P. africana* on the test
225 Organisms
226

Organisms	Minimum Inhibitory Concentration (mg/mL)	Control (mg/mL)
Salmonella typhi	12.5	50
Streptococcus pyogenes	12.5	50
MRSA	25.0	50
Klebsiella pneumoniae	25.0	50
Pseudomonas aeruginosa	50.0	50
Candida albicans	25.0	50

227
228
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230 **Table 3:** Minimum Bactericidal and Fungicidal Concentration of Methanol Extract of *P.*
231 *africana* on the test Organisms

Organisms	MBC/MFC (mg/mL)
Salmonella typhi	50.0
Streptococcus pyogenes	50.0
MRSA	50.0
Klebsiella pneumoniae	50.0
Candida albicans	50.0

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4.0 Discussion

The determination of phytochemical constituents in identifying the possible therapeutic agents contained in plants, in order to establish the basis for their uses in folklore medical practice is important for the discovery of new active compound [18].

Variety of different natural chemical compounds such as saponins, tannins, alkaloids, terpenoids, cyanoglycosides, oleic, flavonoids and stearic acids have been found to contain antimicrobial properties in plants and plant products [18-20]. Asif et al. (2015) [21] reported that flavonoids are polyphenolic phytocompounds which are found in plants and possess antifungal, antibacterial, anticancer, anti-inflammatory as well as antioxidant properties.
The phytochemical screening of this plant in this study shows the presence of flavonoids, carbohydrates, terpenes, cardiac glycosides, alkaloids and tannin. This is in accordance with the study conducted by Ajiboye et al. (2013) [22], using seed and pod of *Prosopis africana.* The result of this study also showed similar active compounds, which is in agreement with findings of Ogbeba et al. (2017) [6] but different from the findings by Thakur et al. (2014) [23], where tannins, saponins and terpenes were absent in methanol leaf extract of *Prosopis africana*. The study by Udegbunam et al. (2014) [24], showed the absence of tannins in *Prosopis lappacea*. The occurrence of these bioactive compounds in reasonable amount in the leaves of *P. africana* could have been responsible for its characteristic antimicrobial properties. These are recognized to have antibacterial agent and may be utilized traditionally for the treatment of infectious diseases [25].
Antimicrobial analysis of methanol crude leaf extract of *P. africana* exhibited some level of antimicrobial properties against the tested microorganisms to include *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) *Salmonella typhi, Klebsiella pneumoniae* and *Candida albicans* at various concentrations with varied diameters zones of inhibition ranging from 1mm for 29mm. This is similar to Ajiboye et al. (2013) [22] who tested the aqueous and methanol extract of seed and pod (*P. africana)* against fifteen bacteria at a fix concentration of 25mg/mL and recorded zone of inhibition ranging from 5mm to 17mm. However, the study by Dosunmu et al. (2014) [27], showed no activity against *K. pneumoniae.*

The result of this study also showed no activity against all the tested organisms at concentration of 6.25mg/mL. Although, the extract had activity against MRSA at 12.5mg/mL concentration with MIC of 25.0mg/mL. This is very promising because of the facts that further purification may exhibit better activity which could lead to discovery of a new lead against antibiotics resistant *Staphylococcus aureus.* It is also worthy of notice that the extract is also active against *Pseudomonas aeruginosa* at crude MIC of 50.0mg/mL.

5. Conclusion

The methanolic leaf extract of *P. africana* has displayed varied activity against pathogenic microorganisms and could represent candidate of antimicrobial agent against some human pathogenic microbes. Furthermore, the bioactive ingredients

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278 indicated that the plant part have proved its usage in the folkloric medicine for the
279 management of different ailments and could be the basis of alternative anti-infective
280 therapy. Therefore, these findings shall broaden and enhance global data base of the
281 antimicrobial property of the active ingredients present.

282 **6. Limitation and Way Forward of the study**

283 The limitation of this study includes lack of funding to permit us to buy solvents for
284 fractionations which will enable us to obtain a pure compound. Therefore, it is
285 recommended that the Federal Government should encourage our health institutions
286 and other related sectors in funding research.

287 **Conflict of Interest**
288 The authors declared no conflict of interest.

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