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Original Research Article

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Phytochemical analysis and Antimicrobial Susceptibility of Methanol Leaf Extract of *Prosopis Africana* (African mesquite) against Selected Microorganisms.

5

6

Abstract

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

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

62 It is in knowledge domain that antimicrobial resistance is a great challenge to holistic
63 treatment of infectious diseases as a result of either the use of substandard antibiotics,
64 misuse or over prescription [9]. There is need for searching and discovering of new
65 lead principles that will be effective, safe, readily available and cost effective for these
66 challenges would go a long way of solving such challenges. Therefore, this study was
67 aimed in determining the phytochemical and antimicrobial properties of *P. africana*
68 towards the development of new antimicrobial agent.
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71 **2.0 Materials and Methods**

72 **2.1 Plant Sample Collection and Identification**

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

78 **2.2 Preparation and Preservation of Plant Material**

79 The leaves were properly washed under clean-running tap water to remove the dirt and
80 air dried at room temperature for a week. The dried leaves were pulverized into powder
81 with clean wooden pestle and mortar, and sieved. The pulverized sample was stored in
82 a clean plastic container, properly labeled and tightly covered at 37°C prior for further
83 analysis [11].

84 **2.3 Extraction of Crude Extract**

85

86 One hundred grams (100g) of the pulverized leave of *P. africana* was accurately
87 weighed and subjected to cold maceration in 500mL of absolute methanol for 72 h at
88 laboratory temperature. The macerated extract was filtered using Whatman No.1 filter
89 paper. The extraction was repeated for the maximum extraction of the active
90 ingredients and to also obtain reasonable yield (crude extract). The filtrate was dried
91 using water bath at 45°C until all the solvent evaporated out [11,12].

92 The percentage yield of the crude extract (PYCE) was calculated using the formula by
93 [11].

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

94

95

96 **2.4 Phytochemical Screening of the Extract**

97 The qualitative phytochemical screening of the leaves of *P. africana* was carried out in
98 Pharmacognosy unit, Department of Medicinal Plant Research and Traditional
99 Medicine, NIPRID, Idu-Abuja. The standard methods illustrated by [12,13,14] were
100 adopted to test for the presence of carbohydrates, flavonoids, phenols/tannins,
101 saponins, terpenes, steroids, alkaloids and cardiac glycosides.

102 **2.5 The Test Microorganisms**

103

104 Antimicrobial activity of methanol extract of leaves of *P. africana* was investigated
105 against five bacterial isolates and one fungal isolate which were obtained from Vaccine
106 Discovery and Research Laboratory, Centre for Genetic Engineering and
107 Biotechnology, Federal University of Technology, Minna, Nigeria. The bacteria strains
108 used for the study include *Salmonella typhi, Klebsiella pneumoniae*, *Streptococcus*
109 *pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus*

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110 (MRSA) and the fungi used for the study was *Candida albicans*. The tested bacteria
111 were maintained on Nutrient agar at 37°C for 24 h and *Candida albicans* on Potatoes
112 Dextrose Agar at 30°C for 48-72 h.

113 **2.6 Inoculum Preparation**

114 A loopful of isolated colonies were inoculated into 4 mL sterile Mueller-Hinton Broth
115 (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and incubated at
116 37°C for 2 h. The turbidity of actively growing microbial suspensions were adjusted with
117 freshly prepared MHB and SDB using $BaSO_4$ turbidity standard to match turbidity
118 standard of 0.5 McFarland. This turbidity was equivalent to approximately $1.5x10^8$
119 CFU/mL cells for bacteria, and $1.5x10^7$ spores/mL for the fungi strain. The grown
120 suspension was used for further testing.

121 **2.7 Preparation of Crude Extract**

122

123 For the preparation of the stock solution, 0.5g of the crude extract was accurately
124 weighed using analytical weighing balance into a sterile tube containing 1mL of 2%
125 Dimethylsulfoxide (DMSO). This was vortexed to allow the extract to completely
126 dissolve and 9mL of sterile distilled water was added to give final extract concentration
127 of 50mg/mL. Double fold dilutions was carried out to give extract concentrations of
128 25mg/mL, 12.5mg/mL and 06.25mg/mL using sterile distilled water respectively.

129 **2.8 *In-vitro* Antimicrobial Susceptibility Assay of the Extract**

130

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

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

159 **2.9 Determination of Minimum Inhibitory Concentration (MIC)**

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

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

180 **2.10 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC**
181 **and MFC)**
182

183 The MBC and MFC were determined from the Minimum Inhibitory Concentration (MIC)
184 result by subculturing from the wells that shows no any sign of turbidity in the MIC test
185 and streak on the freshly prepared MHA and SDA plates and incubate at 37^oC for 24 to
186 48 h and the plates were checked for the present or absent of the growth [16,17].

187 **3.0 Results**
188

189 **3.1 Phytochemical Constituents**
190

191 Qualitative biological active compounds of the methanolic crude extract disclosed the
192 occurrence of carbohydrates, flavonoids, phenols/tannins, saponins, terpenes, steroids,
193 cardiac glycosides and alkaloids respectively as demonstrated in table 1 below.

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

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

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218 **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	+

219 Key: + = Present - = Absent

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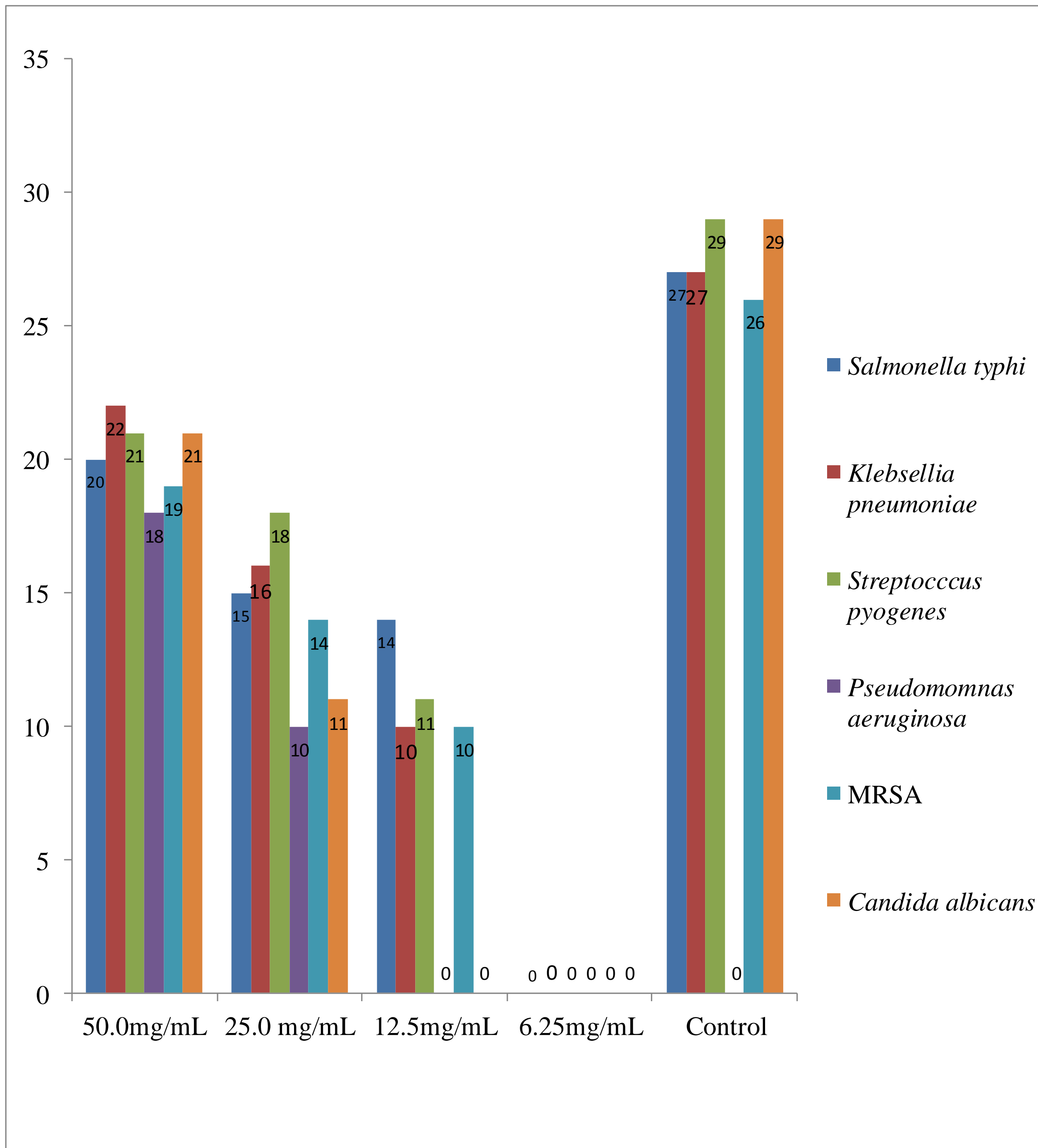

220 **Fig. 1**: Mean zone of inhibition of methanolic extract of *P. africana* on the test organisms

221 (mm).

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223

224

225 **Table 2:** Minimum Inhibitory Concentration of Methanol Extract of *P. africana* on the test
226 Organisms

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

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228

229

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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232 **4.0 Discussion**

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

237 Variety of different natural chemical compounds such as saponins, tannins, alkaloids,
238 terpenoids, cyanoglycosides, oleic, flavonoids and stearic acids have been found to
239 contain antimicrobial properties in plants and plant products [18] [19] [20]. In 2015, Asif
240 and co-workers reported that flavonoids are polyphenolic phytocompounds which are
241 found in plants and possess antifungal, antibacterial, anticancer, anti-inflammatory as
242 well as antioxidant properties [21].
243 The phytochemical screening of this plant in this study shows the presence of
244 flavonoids, carbohydrates, terpenes, cardiac glycosides, alkaloids and tannin. This is in
245 accordance with the study conducted by [22], using seed and pod of *Prosopis Africana.*
246 The result of this study also showed similar active compounds, which is in agreement
247 with findings of [6] but different from the findings by [23], where tannins, saponins and
248 terpenes were absent in methanol leaf extract of *Prosopis africana*. The study by [24],
249 showed the absence of tannins in *Prosopis lappacea*. The occurrence of these bioactive
250 compounds in reasonable amount in the leaves of *P. africana* could have been
251 responsible for its characteristic antimicrobial properties. These are recognized to have
252 antibacterial agent and may be utilized traditionally for the treatment of infectious
253 diseases [25].
254 Antimicrobial analysis of methanol crude leaf extract of *P. africana* exhibited some level
255 of antimicrobial properties against the tested microorganisms to include *Streptococcus*
256 *pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus*
257 (MRSA) *Salmonella typhi, Klebsiella pneumoniae* and *Candida albicans* at various
258 concentrations with varied diameters zones of inhibition ranging from 1mm for 29mm.
259 This is similar to [22] who tested the aqueous and methanol extract of seed and pod (*P.*
260 *Africana)* against fifteen bacteria at a fix concentration of 25mg/mL and recorded zone
261 of inhibition ranging from 5mm to 17mm. However, the study by [27], showed no activity
262 against *K. pneumoniae.*

263 The result of this study also showed no activity against all the tested organisms at
264 concentration of 6.25mg/mL. Although, the extract had activity against MRSA at
265 12.5mg/mL concentration with MIC of 25.0mg/mL. This is very promising because of the
266 facts that further purification may exhibit better activity which could lead to discovery of
267 a new lead against antibiotics resistant *Staphylococcus aureus.* It is also worthy of
268 notice that the extract is also active against *Pseudomonas aeruginosa* at crude MIC of
269 50.0mg/mL.

270 **5. Conclusion**

271

272 The methanolic leaf extract of *P. africana* has displayed varied activity against
273 pathogenic microorganisms and could represent candidate of antimicrobial agent
274 against some human pathogenic microbes. Furthermore, the bioactive ingredients
275 indicated that the plant part have proved its usage in the folkloric medicine for the

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management of different ailments and could be the basis of alternative anti-infective therapy. Therefore, these findings shall broaden and enhance global data base of the antimicrobial property of the active ingredients present.

6. Limitation and Way Forward of the study

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

Conflict of Interest

The authors declared no conflict of interest.

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