

1  
2

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.

31

## 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.

## 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].

$$92 \text{ PYCE} = \frac{\text{Mass of the crude extract obtained}}{\text{Mass of the pulverized plant sample}} \times 100$$

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

114 37°C for 2 h. The turbidity of actively growing microbial suspensions were adjusted with  
115 freshly prepared MHB and SDB using BaSO<sub>4</sub> turbidity standard to match turbidity  
116 standard of 0.5 McFarland. This turbidity was equivalent to approximately 1.5x10<sup>8</sup>  
117 CFU/mL cells for bacteria, and 1.5x10<sup>7</sup> 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<sup>8</sup> CFU/mL cells for bacteria, and 1.5x10<sup>7</sup>  
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)**

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)**

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**

### 187 **3.1 Phytochemical Constituents**

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.

192

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).

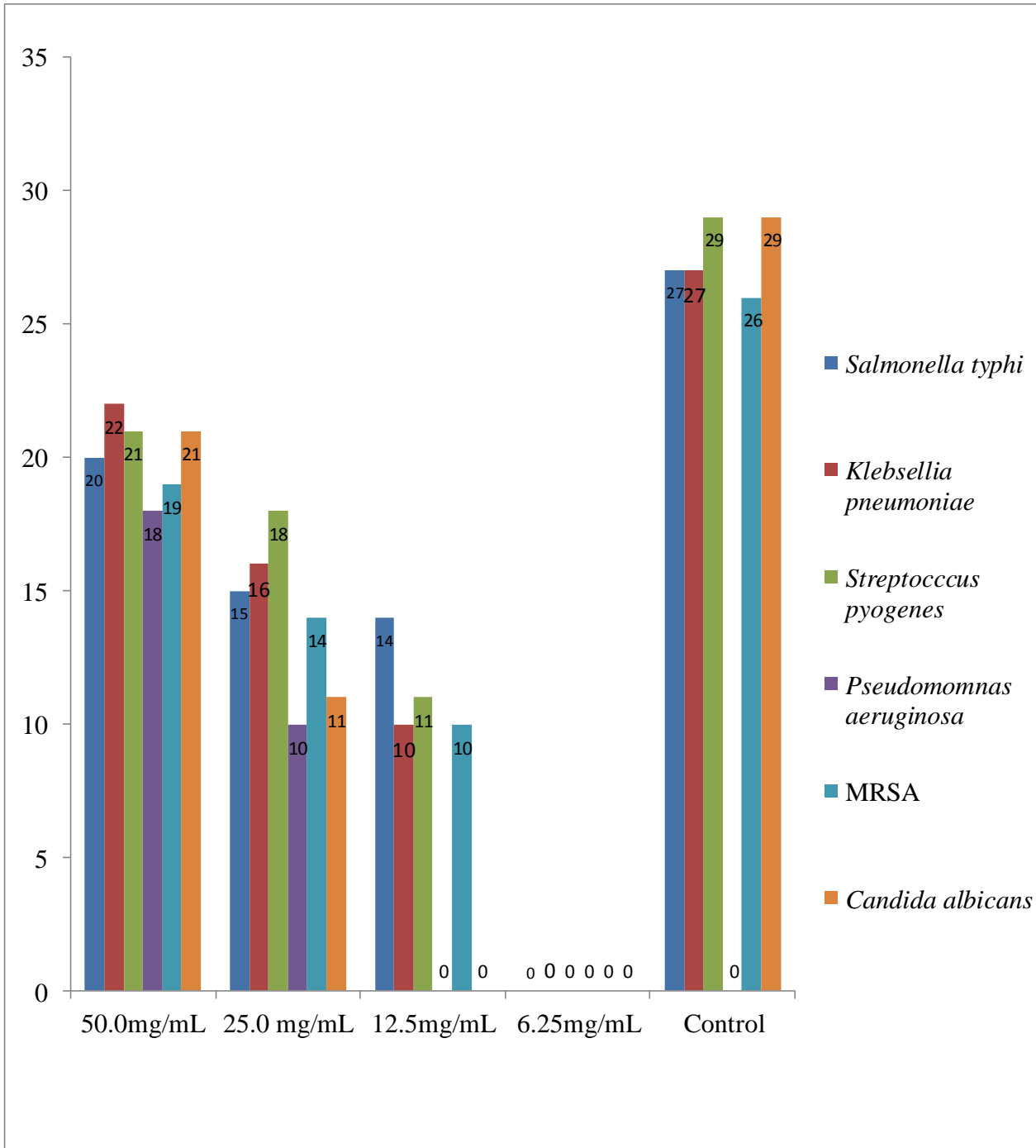
210  
211  
212  
213  
214  
215

UNDER PEER REVIEW

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



218 **Fig. 1:** Mean zone of inhibition (mm) of methanolic extract of *P. africana* on the test  
 219 organisms  
 220

221

222

223



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)
<i>Salmonella typhi</i>	12.5	50
<i>Streptococcus pyogenes</i>	12.5	50
MRSA	25.0	50
<i>Klebsiella pneumoniae</i>	25.0	50
<i>Pseudomonas aeruginosa</i>	50.0	50
<i>Candida albicans</i>	25.0	50

227  
 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)
<i>Salmonella typhi</i>	50.0
<i>Streptococcus pyogenes</i>	50.0
MRSA	50.0
<i>Klebsiella pneumoniae</i>	50.0
<i>Candida albicans</i>	50.0

232  
 233

#### 234 4.0 Discussion

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

239 Variety of different natural chemical compounds such as saponins, tannins, alkaloids,  
240 terpenoids, cyanoglycosides, oleic, flavonoids and stearic acids have been found to  
241 contain antimicrobial properties in plants and plant products [18-20]. Asif et al. (2015)  
242 [21] reported that flavonoids are polyphenolic phytochemicals which are found in  
243 plants and possess antifungal, antibacterial, anticancer, anti-inflammatory as well as  
244 antioxidant properties.

245 The phytochemical screening of this plant in this study shows the presence of  
246 flavonoids, carbohydrates, terpenes, cardiac glycosides, alkaloids and tannin. This is in  
247 accordance with the study conducted by Ajiboye et al. (2013) [22], using seed and pod  
248 of *Prosopis africana*. The result of this study also showed similar active compounds,  
249 which is in agreement with findings of Ogbeba et al. (2017) [6] but different from the  
250 findings by Thakur et al. (2014) [23], where tannins, saponins and terpenes were absent  
251 in methanol leaf extract of *Prosopis africana*. The study by Udegbumam et al. (2014)  
252 [24], showed the absence of tannins in *Prosopis lappacea*. The occurrence of these  
253 bioactive compounds in reasonable amount in the leaves of *P. africana* could have been  
254 responsible for its characteristic antimicrobial properties. These are recognized to have  
255 antibacterial agent and may be utilized traditionally for the treatment of infectious  
256 diseases [25].

257 Antimicrobial analysis of methanol crude leaf extract of *P. africana* exhibited some level  
258 of antimicrobial properties against the tested microorganisms to include *Streptococcus*  
259 *pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus*  
260 (MRSA) *Salmonella typhi*, *Klebsiella pneumoniae* and *Candida albicans* at various  
261 concentrations with varied diameters zones of inhibition ranging from 1mm for 29mm.  
262 This is similar to Ajiboye et al. (2013) [22] who tested the aqueous and methanol extract  
263 of seed and pod (*P. africana*) against fifteen bacteria at a fix concentration of 25mg/mL  
264 and recorded zone of inhibition ranging from 5mm to 17mm. However, the study by  
265 Dosunmu et al. (2014) [27], showed no activity against *K. pneumoniae*.

266 The result of this study also showed no activity against all the tested organisms at  
267 concentration of 6.25mg/mL. Although, the extract had activity against MRSA at  
268 12.5mg/mL concentration with MIC of 25.0mg/mL. This is very promising because of the  
269 facts that further purification may exhibit better activity which could lead to discovery of  
270 a new lead against antibiotics resistant *Staphylococcus aureus*. It is also worthy of  
271 notice that the extract is also active against *Pseudomonas aeruginosa* at crude MIC of  
272 50.0mg/mL.

#### 273 5. Conclusion

274  
275 The methanolic leaf extract of *P. africana* has displayed varied activity against  
276 pathogenic microorganisms and could represent candidate of antimicrobial agent  
277 against some human pathogenic microbes. Furthermore, the bioactive ingredients

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.

289

## 290 **References**

291

- 292 [1] Paul C, Arnold JV, Dirk VB, Louis M. Anti-infective potential of natural products:  
293 How to develop a stronger *in vitro* proof-of-concept. Journal of  
294 Ethnopharmacology. 2006;106:290-302.  
295
- 296 [2] Zial-UL-haq M, Ahmed S, Bukhari AS, Amarowicz R, Ercisli S, Jaafar HZE.  
297 Compositional studies and Biological activities of some mash bean (*Vigna mungo*  
298 **(L.)** Hepper) Cultivars commonly consumed in Pakistan. Biological Research.  
299 2014; 47:1-14.  
300
- 301 [3] Ruchika S, Swarnjeet K. Antimicrobial and phytochemical screening of  
302 Trikota-Traditional food of Western Rajasthan. Indian Journal of Traditional  
303 Knowledge. 2017;16:270-276.  
304
- 305 [4]. Nair R, Chanda S. Antimicrobial activity of *Terminalia catappa*, *Manilkara*  
306 *zapota* and *Piper betel* leaf extract. Indian Journal of Pharmaceutical  
307 Sciences. 2008; 70: 390-393.
- 308 [5]. Mann A, Mohammed S, Adeyanju V. Antimicrobial activity of the root bark and  
309 leaf extracts of the *Capparis brasili* DC. Journal of Pharmaceutical and  
310 Biomedical Sciences. 2012; 3:1-5.  
311
- 312 [6] Ogbeba J, Iruolaje FO, Dogo BA. Antimicrobial Efficacy of *Guiera*  
313 *senegalensis* and *Prosopis africana* Leave Extract on some Bacterial Pathogens.  
314 European Journal of Biology and Medical Science Research. 2017; 5:27-36.
- 315 [7] Dhiman A, Nanda A, Ahmed S, Narasimham B. In vitro antimicrobial status of  
316 methanolic extract of *Citrus sinensis* Linn. Fruit peel. Chronicle of Young  
317 Scientists. 2012; 3:204-208.

- 318  
319 [8] Agboola DA. *Prosopis africana* (Mimosaceae): Stem, Roots, and Seeds in the  
320 Economy of the savanna areas of Nigeria. *Economic Botany*. 2004; 58(Suppl  
321 1):S34. <https://doi.org/10.1663/0013-0001>.  
322
- 323 [9] Abah JO, Agunu A, Ibrahim G, Halilu ME, Abubakar MS. Development of Quality  
324 Standards of *Prosopis africana* (Guill. & Perr.) Taub. Stem Bark. *Journal of*  
325 *Biology, Agriculture and Healthcare*. 2015; 6:10–17.  
326
- 327 [10] Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibé HC, Onyika EO,  
328 Anigo KM, Abu EA, James DB, Njoku GC, Sallau AB. Indigenous Knowledge  
329 system for treatment of Trypanosomosis in Kaduna State of Nigeria. *Journal of*  
330 *Ethnopharmacology*. 2002; 79:279-82.
- 331 [11] Treas GE, Evans WC. *Pharmacognosy*, 11<sup>th</sup> edition, Bailliere Tindall, London.  
332 1989; 45-50.
- 333 [12] Banso A, Adeyemo SO. Phytochemical screening and antimicrobial Assessment  
334 of *Abutilon mauritanum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistritz*.  
335 2006; 18:39-44.
- 336 [13] Harborne JB. *Phytochemical Methods*. Chapman and Hall Limited, London.  
337 1973; 49-188.
- 338 [14] Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books  
339 Limited., Ibadan, Nigeria. 1993; 191-289.
- 340 [15] Bauer AW, Kirby WM, Sherris JC, Turk M. Antibiotic susceptibility testing by  
341 standard single disk method. *American Journal of Clinical Pathology*. 1966;  
342 45:493-496.  
343
- 344 [16] NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that  
345 grow aerobically. Approved standard. 2000; 5th ed. NCCLS document M7-A5.  
346 NCCLS, Wayne, Pa.  
347
- 348 [17] Klacnik A, Piskernik SŠ, Barbara J, Možinav SS. Evaluation of diffusion and  
349 dilution methods to determine the antibacterial activity of plant extracts. *Journal*  
350 *of Microbiological Methods*. 2010; 81:121-126.  
351
- 352 [18] Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal Plants and  
353 Natural Products. *Industrial Journal of Pharmacology*. 2000; 32:S81- S118.  
354
- 355 [19] Abdelrahman HF, Skaug N, Whyatt A. Volatile compounds in crude  
356 *Salvadora persica* extracts, *Pharmaceutical Biology*. 2003; 41:392-404.
- 357 [20] Odozi BE, Ibeh IN, Odozi PI, Osakwe AA, Otoikhian CSO. Antimicrobial Activity  
358 of Aqueous and Methanol Extract of *Prosopis africana* on Selected Bacteria

- 359 Isolates. Indo-American Journal of Life Science and Biotechnology. 2014;  
360 2:2347-2243.
- 361 [21] Asif A, Muhammmad K, Hammed S. Antibacterial Activity of Flavonoids  
362 against Methicillin-resistant *Staphylococcus aureus*. Journal of Theoretical  
363 Biology. 2015; 205:231 – 236.
- 364 [22] Ajiboye AA, Agboola DA, Fadimu OY, Afolabi AO. Antibacterial, Phytochemical  
365 and Proximate Analysis of *Prosopis africana* (Linn.) Seed and Pod Extract.  
366 FUTA Journal of Research in Sciences. 2013: 1:101-109.
- 367 [23] Thakur R, Singh R, Saxena P, Mani A. Evaluation of Antibacterial Activity of  
368 *Prosopis juliflora* (SW.) DC. Leaves. African Journal of Traditional Complement  
369 and Alternative Medicine. 2014; 11:182-188.
- 370 [24] Udegbumam OS, Udegbumam IR, Muogbo CC, Anyanwu UM, Nwaehujor CC.  
371 Wound Healing and Antibiotic properties of *Pupalia lappacea* Juss in Rats. BMC  
372 Complementary and Alternative Medicine. 2014; 14:157.  
373 <http://www.biomedcentral.com/1472-6882/14/157>
- 374 [25] Usman H, Osuji JC. Phytochemical and In vitro antimicrobial Assay of the leaf  
375 extract of *Newbouldia leavis*. African Journal of Traditional Products. 2007;  
376 4:476-480.
- 377 [26] Kolapo AAL, Okunade MB, Adejumobi JA, Ogundiya MO. Phytochemical  
378 Composition and Antimicrobial Activity of *Prosopis africana* Against Some  
379 Selected Oral Pathogens. World Journal of Agricultural Sciences. 2009; 5:90-  
380 93.
- 381 [27] Dosunmu OO, Oluwaniyi OO, Awolola GV, Oyedeji OO. Nutritional  
382 Composition and Antimicrobial Properties of Three Nigerian Condiments.  
383 Nigerian Food Journal. 2014; 30:43-52.
- 384  
385  
386  
387  
388  
389