



***In-vitro* Antimicrobial Susceptibility and Phytochemical Constituents of Methanol Leaf Extract of *Prosopis africana* against Some Selected Microorganisms**

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Authors' contributions

This research work was carried out in collaboration among all authors. Author AAR did the conceptualization, proposal writing, discussion/choice of the protocol, plant specimen collection, performed the laboratory experiments (bench work) and writing up of manuscript for publication. Author KZJ did the literature researches, proposal writing proof reading and supervised the bench work. Author MSB carried out literature researches, discussion/choice of the protocol, plant specimen collection, performed the laboratory experiments (bench work), analyzed the data and made the necessary corrections. Author YY was involved in plant specimen collection, performed the laboratory experiments (bench work), designed and managed the analyses of the study. Author IK managed the literature searches and proof reading of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The idea that certain plants had healing potential was known long before human beings discovered the existence of pathogens.

Methodology: The crude methanolic leaf extract of *Prosopis africana* was assayed for antimicrobial potency using Agar-well diffusion technique against *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Methicillin-Resistant

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Staphylococcus aureus (MRSA) and *Candida albicans*. Qualitative phytochemical screening was also carried out.

Results: The results of the antimicrobial screening showed antimicrobial potency against the test isolates with various degrees of zone of inhibition which varied between 10 mm – 22 mm. The highest zone was reported against *Klebsiella pneumoniae* (22 mm), followed by *Streptococcus pyogenes* and *Candida albicans* (21 mm), *Salmonella typhi* (20 mm), MRSA (19 mm) and then *Pseudomonas aeruginosa* (18 mm). Chloramphenicol and Fluconazole are used as reference standard and their zones of inhibitions ranged from 26 mm – 29 mm. The Minimum Inhibitory Concentration (MIC) of the extract ranged between 12.50 mg/mL – 50.00 mg/mL whilst the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the extract were at 50.00 mg/mL. The result of phytochemical screening revealed the presence of carbohydrates, flavonoids, phenols/tannins, saponins, terpenes, steroids, cardiac glycosides and alkaloids as secondary metabolites.

Conclusion: The findings clearly showed that methanolic leaf extract of *P. africana* has proved its use in folklore as an alternative antimicrobial agent and further research can lead to isolation of a new lead of medical importance.

Keywords: Antimicrobial susceptibility; *Prosopis africana*; phytochemical constituents; 96-well microplates; MIC; MBC; MFC.

1. INTRODUCTION

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

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

Prosopis africana (Guill. & Perr.) Taub. belongs to family fabaceae. It is a renowned and versatile

tree of immense economic value amid the rural communities in the Guinea savanna of Nigeria. It is mostly found in savanna countries of Africa like Senegal and Nigeria [8]. Literature gives an account of its uses as folk medicines for several ailment and virtually all its parts are of medicinal value. Abah et al. [9] reported that the stem bark is used as remedies for dysentery, gonorrhoea, bronchitis and skin diseases. In Niger State of Nigeria, the twigs, leaves, bark, and secondary roots are used for treatment and relieve of typhoid fever, dental decay, malaria as well as stomach cramps while, Atawodi et al. [10] attested that the bark and root decoctions are utilized for the treatment of Trypanosomiasis in cattle and on lesions as a lotion.

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

2. MATERIALS AND METHODS

2.1 Plant Sample Collection and Identification

The leaves of *P. africana* were collected in Bida, Niger State, Nigeria. The voucher

specimen was prepared, the plant was identified and voucher specimen was deposited in herbarium unit of the Department of Medicinal Plant Research and Traditional Medicine (MPR & TM), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

2.2 Preparation and Preservation of Plant Material

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

2.3 Extraction of Crude Extract

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

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

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

2.4 Phytochemical Screening of the Extract

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

2.5 The Test Microorganisms

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

2.6 Inoculum Preparation

A loopful of isolated colonies were inoculated into 4 mL sterile Mueller-Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and incubated at 37°C for 2 h. The turbidity of actively growing microbial suspensions were adjusted with freshly prepared MHB and SDB using BaSO₄ turbidity standard to match turbidity standard of 0.5 McFarland. This turbidity was equivalent to approximately 1.5x10⁸ CFU/mL cells for bacteria, and 1.5x10⁷ spores/mL for the fungi strain. The grown suspension was used for further testing.

2.7 Preparation of Crude Extract

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

2.8 In-vitro Antimicrobial Susceptibility Assay of the Extract

Susceptibility test of the extract against the isolates were determined in the Microbiology Laboratory, Ibrahim Badamasi Babangida University Lapai in Niger State, Nigeria using Kirby-Bauer agar diffusion method according to

NCCLS standards [15,16]. The Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were used for the antimicrobial activity test. About 100 μL of MHB and SDB cultures containing 0.5 McFarland equivalents to approximately 1.5×10^8 CFU/mL cells for bacteria, and 1.5×10^7 spores/mL for fungi strain were dispensed into empty sterile Petri dishes using micropipettes. Twenty-three millilitres (23 mL) of sterilized MHA and SDA maintained between 50 – 45°C was added to the appropriate Petri dishes and rocked gently for even distribution of the organisms under aseptic condition and allowed to gel under safety hood for 1 h. On each of the plates containing bacteria isolates. Five wells of 8 mm in diameter were made on the agar plates using sterile metallic cork borer and labelled properly. The base of the wells was sealed with 30 μL of MHA and SDA. Thereafter, 200 μL of different concentrations of the extract were carefully and aseptically added with the aid of micropipette into each well and left in the safety hood for 2 h for proper diffusion of the extracts into the agar and then incubated at 37°C for 24 h for bacteria. The same procedure was repeated for fungi strain and incubated at 25°C for 48 h for fungi. The experiment was set up in duplicates. The plates were observed for activity and zones of inhibitions were measured and recorded as mean zone of inhibition. The diameter of each zone was accurately measured with a spotless and translucent ruler in millimetre (mm) in vertical and horizontal manner and mean was determined.

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

2.9 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) value of the extract of *P. africana* leaves extract was determined by microdilution broth method in 96-well microplates [17]. Chloramphenicol (Fidson, Nigeria) and Fluconazole (Pfizer, UK) were used as the

standard drug for bacteria and fungi at stock concentration of 50 $\mu\text{g/ml}$. Controls of sterility for the Mueller-Hinton nutrient broth, control culture (inoculum), Chloramphenicol, Fluconazole, crude extract and DMSO were carried out. The microwell plates were closed and incubated aerobically at 37°C for 24 h. Thereafter, 50 μL of tetrazolium dye was applied into each well with 2 h further incubation at 37°C and colour change was observed. Any well with reddish-pink colour signifies the microbial growth, which was noted and documented as positive (MIC). All assays were carried out in triplicate.

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

2.10 Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

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

3. RESULTS

3.1 Phytochemical Constituents

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

3.2 Antimicrobial Activity

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

3.3 Minimum Inhibitory and Bactericidal Concentrations of the Crude Extract against Sensitive Organisms

Minimum Inhibitory Concentration (MIC) was carried out on all the isolates where *Salmonella typhi* and *Streptococcus pyogenes* had MIC of 12.50 mg/mL. MRSA, *Klebsiella pneumoniae* and *C. albicans* had MIC of 25 mg/mL while the MIC

of *Pseudomonas aeruginosa* was at 50 mg/mL (Table 2). The MBC of *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, MRSA were at 50 mg/mL and *C. albicans* had the MFC of 50 mg/mL (Table 3).

Table 1. 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	+
Cardiac glycosides	Wagner's	+
	Keller-Kilani	+

Key: + = Present; - = Absent

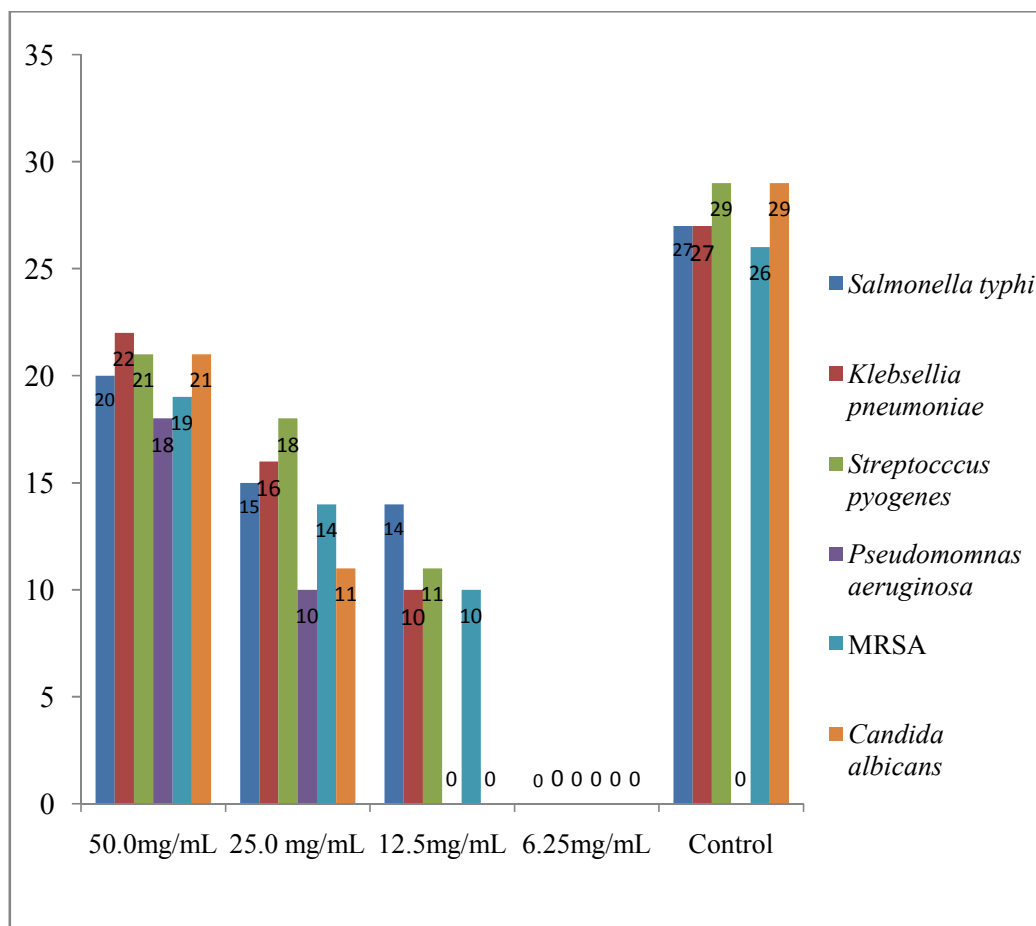


Fig. 1. Mean zone of inhibition(mm) of methanolic extract of *P. africana* on the test organisms

Table 2. Minimum inhibitory concentration of methanol extract of *P. africana* on the test

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

Table 3. Minimum bactericidal and fungicidal concentration of methanol extract of *P. 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

4. 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. [21] reported that flavonoids are polyphenolic phytochemicals 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. [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. [6] but different from the findings by Thakur et al. [23], where tannins, saponins and terpenes were absent in methanol leaf extract of *Prosopis africana*. The study by Udegbonam et al. [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 1 mm for 29 mm. This is similar to Ajiboye et al. [22] who tested the aqueous and methanol extract of seed and pod (*P. africana*) against fifteen bacteria at a fix concentration of 25 mg/mL and recorded zone of inhibition ranging from 5 mm to 17mm. However, the study by Dosunmu et al. [27], showed no activity against *K. pneumoniae*.

The result of this study also showed no activity against all the tested organisms at concentration of 6.25 mg/mL. Although, the extract had activity against MRSA at 12.5 mg/mL concentration with MIC of 25.0 mg/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 50.0 mg/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 indicated that the plant part have proved its usage in the folkloric medicine for the 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Paul C, Arnold JV, Dirk VB, Louis M. Anti-infective potential of natural products: How to develop a stronger *in vitro* proof-of-concept. *Journal of Ethnopharmacology*. 2006;106:290-302.
- Zial-UL-haq M, Ahmed S, Bukhari AS, Amarowicz R, Ercisli S, Jaafar HZE. Compositional studies and biological activities of some mash bean (*Vigna mungo* (L.) Hepper) Cultivars commonly consumed in Pakistan. *Biological Research*. 2014;47:1-14.
- Ruchika S, Swarnjeet K. Antimicrobial and phytochemical screening of trikuta-traditional food of western Rajasthan. *Indian Journal of Traditional Knowledge*. 2017;16:270-276.
- Nair R, Chanda S. Antimicrobial activity of *Terminalia catappa*, *Manilkara zapota* and *Piper betel* leaf extract. *Indian Journal of Pharmaceutical Sciences*. 2008;70:390-393.
- Mann A, Mohammed S, Adeyanju V. Antimicrobial activity of the root bark and leaf extracts of the *Capparis brasii* DC. *Journal of Pharmaceutical and Biomedical Sciences*. 2012;3:1-5.
- Ogbeba J, Iruolaje FO, Dogo BA. Antimicrobial efficacy of *Guiera senegalensis* and *Prosopis africana* leave extract on some bacterial pathogens. *European Journal of Biology and Medical Science Research*. 2017;5:27-36.
- Dhiman A, Nanda A, Ahmed S, Narasimham B. *In vitro* antimicrobial status of methanolic extract of *Citrus sinensis* Linn. Fruit peel. *Chronicle of Young Scientists*. 2012;3:204-208.
- Agboola DA. *Prosopis Africana* (Mimosaceae): Stem, roots, and seeds in the economy of the savanna areas of Nigeria. *Economic Botany*. 2004; 58(Suppl 1):S34. Available:https://doi.org/10.1663/0013-0001
- Abah JO, Agunu A, Ibrahim G, Halilu ME, Abubakar MS. Development of quality standards of *Prosopis africana* (Guill. & Perr.) Taub. Stem Bark. *Journal of Biology, Agriculture and Healthcare*. 2015;6:10–17.
- Atawodi SE, Ameh DA, Ibrahim S, Andrew JN, Nzelibe HC, Onyika EO, Anigo KM, Abu EA, James DB, Njoku GC, Sallau AB. Indigenous knowledge system for treatment of *Trypanosomosis* in Kaduna State of Nigeria. *Journal of Ethnopharmacology*. 2002;79:279-82.
- Treas GE, Evans WC. *Pharmacognosy*, 11th edition, Bailliere Tindall, London. 1989;45-50.
- Banso A, Adeyemo SO. Phytochemical screening and antimicrobial assessment of *Abutilon mauritanum*, *Bacopa monnifera* and *Datura stramonium*. *Biokemistritz*. 2006;18:39-44.
- Harborne JB. *Phytochemical methods*. Chapman and Hall Limited, London. 1973; 49-188.
- Sofowora A. *Medicinal plants and traditional medicine in Africa*. Spectrum Books Limited. Ibadan, Nigeria. 1993;191-289.
- Bauer AW, Kirby WM, Sherris JC, Turk M. Antibiotic susceptibility testing by standard single disk method. *American Journal of Clinical Pathology*. 1966;45:493-496.

16. NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. 5th ed. NCCLS document M7-A5. NCCLS Wayne, Pa; 2000.
17. Klacnik A, Piskernik SŠ, Barbara J, Možinav SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods*. 2010; 81:121-126.
18. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products. *Industrial Journal of Pharmacology*. 2000;32:S81-S118.
19. Abdelrahman HF, Skaug N, Whyatt A. Volatile compounds in crude *Salvadora persica* extracts. *Pharmaceutical Biology*. 2003;41:392-404.
20. Odozi BE, Ibeh IN, Odozi PI, Osakwe AA, Otoikhian CSO. Antimicrobial activity of aqueous and methanol extract of *Prosopis africana* on selected bacteria isolates. *Indo-American Journal of Life Science and Biotechnology*. 2014;2:2347-2243.
21. Asif A, Muhammad K, Hamed S. Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus*. *Journal of Theoretical Biology*. 2015;205: 231–236.
22. Ajiboye AA, Agboola DA, Fadimu OY, Afolabi AO. Antibacterial, phytochemical and proximate analysis of *Prosopis africana* (Linn.) seed and pod extract. *FUTA Journal of Research in Sciences*. 2013;1:101-109.
23. Thakur R, Singh R, Saxena P, Mani A. Evaluation of antibacterial activity of *Prosopis juliflora* (SW.) DC. leaves. *African Journal of Traditional Complement and Alternative Medicine*. 2014;11:182-188.
24. Udegbumam OS, Udegbumam IR, Muogbo CC, Anyanwu UM, Nwaehujor CC. Wound healing and antibiotic properties of *Pupalia lappacea* Juss in rats. *BMC Complementary and Alternative Medicine*. 2014; 14:157. Available:<http://www.biomedcentral.com/1472-6882/14/157>
25. Usman H, Osuji JC. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia leavis*. *African Journal of Traditional Products*. 2007;4:476-480.
26. Kolapo AAL, Okunade MB, Adejumbi JA, Ogundiya MO. Phytochemical composition and antimicrobial activity of *Prosopis africana* against some selected oral pathogens. *World Journal of Agricultural Sciences*. 2009;5:90-93.
27. Dosunmu OO, Oluwaniyi OO, Awolola GV, Oyedeji OO. Nutritional composition and antimicrobial properties of three Nigerian condiments. *Nigerian Food Journal*. 2014; 30:43-52.

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