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Antimicrobial Activity of Seed Extracts of *Prosopis Africana* on Some Selected Microorganisms

Author's Details: [(1)] James Hamuel Doughari [(2)]Mom Saa-Aondo
Department of Microbiology, Faculty of Life Sciences, Modibbo Adama University, PMB 2076 Yola, Nigeria
***Corresponding author's email address**: jameshamuel@yahoo.com or jameshamuel@mautech.edu.ng Tel: +2347035599712

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Abstract
To investigate the antimicrobial activity of seed of Prosopis afriacana on clinical isolates of Klebsiella pneumoniae, Pseudomonas aeruginosa and Microsporum canis, agar well diffusion method was used. Extraction using various solvents (distilled water, ethanol and methanol) was done by cold maceration method. Qualitative analysis using the various solvent extracts showed the presence of tannins, flavonoids, alkaloids and phenols in all solvent extracts. The quantitative analysis showed that aqueous and ethanol seed extracts were higher in alkaloids (22.20% and 24.49%) respectively, methanol seed extracts was higher in tannin (1300 mg/100g). All the extracts showed antimicrobial activity against the tested organisms with average diameter zone of inhibition ranging between 6.00-18.00 mm. The minimum inhibitory concentration ranged between 100 mg/ml-200 mg/ml, the minimum bactericidal concentration was at 250 mg/ml for the bacterial isolates and the minimum fungicidal concentration was between 200 mg/ml – 250 mg/ml on the fungal isolate. The results obtained in this work showed that Prosopis africana can be useful in treating ailments caused by pathogenic organisms selected in this work.
***Key words**: Antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration*

INTRODUCTION

Plant medicine is a broad category of medicaments that include drugs used in traditional system of medicine, folklore and ethno medical products, and drug discovered from plants that have hitherto, documented therapeutic use. For many decades, herbal therapy formed the foundation for the treatment of many diseases in Africa. Undoubtedly, medicinal plants and drugs derived from them constitute great medical and economically strategic value not only in African continent but also for other parts of the world (Anon, 1985). Plants base natural constituents can be derived from barks, leaves, flowers, roots, fruits, seeds of plants (Gordon and David, 2001) which in most cases contain active components (Jigna *et al.,* 2006).Over the last few decades, the biological and pharmacological potentials of organic substances from many indigenous plants have been well understood. For instances, phenolic compounds have been associated with antimicrobial (Narayana *et al.,* 1999), anti-inflammatory, antiviral and cytotoxic activities (Chhabra *et al.,* 1984). However, the bioactive constituents conferring these properties on many plant species also been implicated in allelopathy (Nazir *et al.,* 2007). Allelopathy has been defined as the effect (s) of one plant on other plants through the release of chemical compounds in the environment (Rice, 1984).

Studies (Okafor *et al.,* 2001; Okafor *et al.,* 2002; Okoli and Iroegbu, 2004; Ofokansi *et al.,* 2005) have given credence to the folkloric claims on the prophylactic and therapeutic effectiveness of some medicinal plants against infectious agents including multidrug resistant ones. Notable among them is *Prosopis africana* (Eze *et al.,* 2010).

The tree of *Prosopis africana* are common in the middle belt and Northern parts of Nigeria and are refered to as "Kiriya" and "Okpehe" in Hausa and Idoma languages (Ajiboye, 2009) and "Gbaaye" in Tiv language respectively. In many areas where the trees are grown or available, the fermented seeds of *P.africana* are used as food condiments, its young leaves and shoot are fodder that is highly sought after towards the end of the dry season (Ajiboye, 2009). Almost all parts of the plant are used in medicine, the leaves in particular for the treatment of headache and toothache as well as various other head ailments. Leaves and the barks are combined to treat rheumatism. Remedies for skin disease, dental caries, fevers and eye washes are obtained from the bark. The roots are diuretics and are used to treat gonorrhea, toothache, stomachache, dysentery, malaria and bronchitis. In Mali, the leaves, bark twigs and roots are used to treat and relieve bronchitis, tooth decay, dysentery, malaria and stomach cramps; in Ghana, boiled roots are served as a poultice for sore throat, root decoctions for toothache and bark as a dressing lotion for wounds or cuts (Orwa, *et al.*, 2009). The purpose of this work is to determine the phytochemical analysis and evaluate the antimicrobial activity of seed extracts of *Prosopis africana* on some selected microorganisms.

MATERIALS AND METHODS

Collection and Identification of Plant Sample

Dried pods containing seeds (Fig 1) of *Prosopis africana* were collected in December 2016, from Mba-Agav District, Gyaruwa,Gwer West Local Government Area of Benue State. The plant was identified in the Department of Plant Science, Modibbo Adama University of Technology, Yola, Adamawa State.



Fig 1. Seeds of *Prosopis africana* (source: Field work 2019),

Plant Processing and Solvent Extraction

The pods containing seeds were broken and the seeds removed and separated from the pod. The seeds were pounded using mortar and pestle into powdered form. Five hundred (500) grams powdered material was weighed and soaked in one and half liters of various solvents (distilled water, ethanol and methanol) in McCartney bottles with intermittent shaking for 24 h. The extracts were then filtered off using sterile filter paper (No 42) into different clean conical flasks and the concentrated extracts were allowed to dry at room temperature. The obtained solvent extracts were reconstituted in 500ml of distilled water with warming and further fractionated by extracting with 400ml of each of methanol, chloroform and n-hexane separately. The various fractions were allowed to dry at room temperature to obtain methanol, chloroform and n-hexane fractions of the various extracts respectively. The various fractions were stored in the refrigerator at 4^{o}C until when needed for susceptibility testing.

Phytochemical Analysis

Qualitative Analysis

The qualitative phytochemical analysis of seed of *P. africana* was carried outs using the standard procedures described by Harborne (1998) and Sofowora (1993).

Test for Saponins (Froting test)
To 5ml of each solvent extracts, 5.0 ml of distilled water was added in a test tube and shaken vigorously. Formation of foam was taken as an indication of saponin. (Harborne, 1998)

Test for Tannins
To 1ml of each solvent extract, 2.0 ml of 5% ferric chloride was added formation of dark blue or greenish black indicated the presence of tannin (Harborne, 1998).

Test for Terpenoids
To 2.0 ml of each solvent extract in a tube, 2.0 ml of chloroform was added the 3ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. Formation of a reddish brown colour interface was indicative of the presence of terpenoids (Harborne, 1998).

Test for Flavonoids
To 2.0 ml of each solvent extracts, few drops of concentrated ammonia (NH_3) was added in a test tube. A yellow colouration was indicative of the presence of flavonoids (Harborne, 1998).

Test for Alkaloids
To 2.0 ml of each solvent extracts, 2.0 ml of concentrated hydrochloride acid (HCl) was added. Then 3.0 ml of Mayer's reagent was added. The presence of light green colouration indicates the presence of alkaloids (Sofowora, 1993).

Test for Glycosides
To 2.0 ml of each solvent extracts, 1ml off glacial acetic and (CH_3CO_2H) and 5% ferric chloride ($FeCl_3$) was added. To these, few drops of concentrated hydrochloride acid (HCl) were added. A brown ring was observed indicating the presence of glycosides (Harborne, 1998).

Test for Steroids
About 2.0 ml of acetic anhydride was added to 5ml of each solvent extract with 2.0 ml sulphuric acid (H_2SO_4). The colour change from violet to blue or green was taken for the presence of steroids (Harborne, 1998).

Test for Phenols
To 1.0 ml of each solvent extracts, 1.0 ml of 10% ferric chloride ($FeCl_3$) was added. The formation a greenish brown or black precipitate or colour indicated the presence of Phenols (Harborne, 1998).

Quantitative Analysis
Saponin Determination
A 5.0 g of each solvent extract was mixed with 50.0 ml of 20% ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C. It was then filtered through whatman fitter paper (No. 42). The residue was re-extracted with 50.0 ml of 20% ethanol and both extracts for each solvent extracts were combined and reduced to about 40.0 ml at 90°C and transferred to a separating funnel where 400 ml of diethyl ester was added and shaken vigorously. Separation was by partitioning during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer became clear. The saponins were extracted with 60.0 ml n-butanol; the combined extracts were washed with aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60°C in the oven and reweighed after cooling in a desiccator. The saponins content was determined by difference and calculated as a percentage of the original sample (Harborne, 1998). The following formula was used in the calculation:

$$\% \text{ Saponins} = \frac{\text{Final weight of sample}}{\text{Total weight of sample}} \times 100.$$

Estimation of Tanins (Folin-Denis method)

About 1.0 g of each sample was dispersed in 10.0 ml distilled water and agitated. This was left to stand for 30 min at room temperature and shaken every 5 min. After 30 min, it was centrifuged and the extract gotten. About 2.5ml of the supernatant extract was dispensed into separate 50.0ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispensed into a separate 50.0ml flask. The absorbance was measured at 250nm and compared to the standard tannic curve obtained by plotting optical density (absorbance) versus tannic acid concentration (Pearson, 1976) (Appendix II). The following formula was used in the calculation:

$$\text{Tannic acid } (\frac{\text{mg}}{100\text{g}}) = \frac{\text{C} \times \text{extracts volume} \times 100}{\text{Aliquote volume} \times \text{weight of sample}}$$

Where C is concentration of tannic acid read on the graph.

Estimation of Terpenoids

About 2.0 g of each sample was weighed and soaked in 50ml of 95% ethanol for 24 hrs. The extract was filtered and the filtrate extracted with petroleum ether (60°C–80°C) and concentrated to dryness. The dried ether extraction was treated as total terpenoids (Ferguson, 1956). The following formula was used in the calculation:

$$\% \text{ Terpenoids} = \frac{\text{Final weight of sample}}{\text{Total weight of sample}} \times 100.$$

Flavonoids Determination

Ten grams (10g) of each solvent extract was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrates were transferred into different crucibles and evaporated into dryness over a water bath and weighed to a constant weight (Bohm and Kocipai - Abyazan, 1994). The following formula was used in the calculation:

$$\% \text{ Flavonoids} = \frac{\text{Final weight of sample}}{\text{Total weight of sample}} \times 100$$

Alkaloids Determination

Five grams (5g) of each sample was weighed into a 250ml beaker and 200ml of 100% acetic acid in ethanol was added and covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to each extract until the precipitin was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid, which was dried and weighed (Harborne, 1998). The following formula was used in the calculation:

$$\% \text{ Alkaloids} = \frac{\text{Final weight of sample}}{\text{Total weight of sample}} \times 100.$$

Glycoside Determination

Glycoside quantitative determination methodology used in this research is that by Amadi *et al.,* (2004). The extract was weighed into a 250 cm^3 round bottom flask and about 200 cm^3 of distilled water was added to one gram of each powder sample in the conical flask and allowed to stand for 2 h for autolysis to occur. Full distillation was carried out in a 250 cm^3 conical flask containing 20 cm^3 of 2.5 % NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Glycoside (100 cm^3), 8 cm^3 of 6 M NH_4OH (ammonium hydroxide), and 2 cm^3 of 5 % KI (potassium iodide) were added to the distillate(s), mixed, and

titrated with 0.02 M $AgNO_3$ (silver nitrate) using a micro burette against a black background. Turbidity which was continuous indicates the end point. The following formula was used in the calculation:

$$\text{Glycoside } (\tfrac{mg}{100g}) = \frac{\text{Titre value } (cm^3) \times 1.08 \times \text{extract volume}}{\text{Aliqote volume } (cm^3) \times \text{sample weight } (g)} \times 100.$$

Steroids Determination

This was determined according to the method of Harborne (1998). Five (5g) grams of sample was hydrolysed by boiling in hydrochloric acid solution for 30 minutes. It was filtered using whatman filter paper (No 42) the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100 ^oC for 5minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed whatman filter paper (No 42) was used to filter the mixture properly. The dry extract was then cooled in a dessicator and reweighed. The process was repeated two more times and an average was obtained. The concentration of steroid was determined and expressed as a percentage thus.

$$\% \text{ Steroids} = \frac{\text{Final weight of sample}}{\text{Total weight of sample}} \times 100.$$

Determination of Phenols

One gram (1g) of the fat free sample was boiled with 50ml of ether for the extraction of phenolic component for 15 min. 5ml of the extract was taken into 50ml flask distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was also added. The samples were made up to the mark and left to react for 30 min. for colour development (Edeogal *et al.,* 2005; Jing-Chung *et al.,* 2007). These were measured at 505nm. These data were used to estimate the total phenolic content using a standard calibration of gallic acid.

Test for Purity of Extract

Both plant extracts fractions were plated on the nutrient agar and incubated at 37^oC for 24 h to ensure purity.

Test Organisms

The test organisms were clinical isolates of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Microsporum canis*. They were obtained from the microbiology laboratory of Specialist Hospital, Yola, Adamawa State Nigeria and reconfirmed using standard procedures before being used for the antimicrobial activity.

Antimicrobial Activity

The antimicrobial activity of the methanol fraction of Aqueous and ethanolic extracts of seed of *Prosopis afriacana* was carried out on the test organisms using agar well diffusion method method of Sen and Batra (2012). Mueller Hinton agar (MHA) was used for bacteria. MHA was seeded with test organisms which were adjusted to 0.5 McFarland turbidity standard according to CLSI (2006) using sterile swab stick. Wells (6mm diameter and 2cm apart) were made in each of the plates using sterile cork borer. Each fraction extract was reconstituted in distilled water to obtain various concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml). Aliquote of 0.1ml (100 µl) of different concentrations of each solvent extracts were added using sterile micropipette into wells and allowed to diffuse at room temperature for 1 h. Ampicillin (10 mg/ml) and distilled water was used as positive and negative controls respectively. The plates were incubated at 37$^\circ C$ for 18-24 h. The diameter of zone of inhibition in millimeter was measured using a ruler. All experiments were carried out in duplicates and readings were taken in 3 different directions and average values recorded.

Antifungal Susceptibility Testing

The antifungal activity was carried out using the pour plate method of Bazie *et al.* (2014) Sauboraud's Dextrose Agar (SDA) supplemented with chloramphenicol and cyclohexamide to prevent the growth of bacteria and saprophytic fungi was used. Five (5) ml of culture suspension of *Microsporum canis* with a concentration of 1×10^6 conidia ml^{-1} was added to 240 ml of SDA which had been previously cooled to 50^oC. Wells (6 mm and 2 cm apart) were made using sterile cork borer and 100 µl (0.1 ml) of each fraction extracts was transferred into the wells using sterile pipette and incubated at $28^{\circ}C$ for 4 days. Griseofulvin (10 mg) and distilled water was used as positive and negative control respectively. The diameter of zone of inhibition around the wells was measured in three different directions and average taken using ruler.

Determination of Minimum Inhibitory Concentration (MIC)

The MICs was determined according to the method of Doughari (2008). Broth cultures of various organisms (Nutrient broth for bacteria and potato dextrose broth for fungi) were prepared and incubated at 37^oC for 18-24 h for bacteria and 28^oC for 72 h for fungi. Nutrient broth and potato dextrose broth were prepared according to the manufacturer's guideline and dispensed into five test tubes for each organism. 1 ml of various plant concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml) was transferred into various test tubes containing sterile broth. Aliquote of standardized *K.pneumoniae* and *P.aeruginosa* were inoculated into the test tubes containing nutrient broth respectively using sterile Pasteur pipette while the standardized fungus (*M. canis*) was inoculated into test tubes containing potato dextrose broth supplemented with chloramphenicol. The various test tubes containing the broth with the various organisms and plants extracts were incubated at 37^oC for 24 h for bacteria and 28^oC for 72 h for fungi. The test tubes were then examined for growth. The lowest concentration without visible growth was taken as the MICs for the various organisms.

Determination of minimum bactericidal Concentration (MBC) and minimum Fungicidal Concentration (MFC)

Nutrient broth and potato dextrose broth culture test tubes with low extract concentration that showed no visible growth from the broth dilution technique above was sub-cultured on nutrient agar (NA) for bacteria and Sabouraud Dextrose Agar (SDA) plates and incubated at 37^oC for 24 h and 28^oC for 72 h. The plates were examined visually for colonies. MBC and MFC were examined at plates with no growth (Doughari, 2008).

RESULTS

Qualitative and Quantitative Analysis of *P.africana* seeds for Phyto-constituents

Result of the qualitative analysis showed that all the solvents extracts of seed have saponin, flavonoids, alkaloids and phenols. It also showed that ethanol seed extract lacked tannin, terpenoids and steroids while methanol seed extracts lacked steroids and the aqueous seed extracts lacked glycosides.

Quantitative analysis of seed extracts of *Prosopis africana* showed that the aqueous and ethanol seed extracts have higher percentages (22.20% and 24.40 % respectively) of alkaloids among the phyto-constituents tested while methanol seed extracts was higher in tannins (1300 mg/100g) (Table 1). Furthermore, the result showed that on the average, the three solvent extracts viz ethanol, methanol and aqueous, have higher percentages of alkaloids (24.40%, 23.40% and 22.20%) (Table 2).

Table 1. Phyto-constituents of seed of *Prosopis Africana*

Phyto-constituents	Composition of components in extracts		
	Ethanol	Methanol	Aqueous
Tannin	-	+	+
Glycosides	+	+	-
Phenols	+	+	+
Saponin	+	+	+
Terpenoid	-	+	+
Flavonoids	+	+	+

Alkaloid	+	+	+
Steroids	-	-	+

Key: + = Present, - = Absent.

Table 2. Composition of phyto-constituents in seed of *P. africana.*

Phyto-constituents	Composition of components in extracts		
	Ethanol	Methanol	Aqueous
Tannin (mg/100g)	-	1300	930
Glycosides (mg/100g)	100	140	-
Phenols (mg/g)	0.77	0.94	0.88
Saponin (%)	21.20	18.20	15.40
Terpenoid (%)	-	4.00	15.50
Flavonoids (%)	8.80	4.90	5.20
Alkaloid (%)	24.40	23.40	22.20
Steroids (%)	-	-	0.80

Key: - = Absent.

Antimicrobial Activity of Seed *Prosopis africana*

Result of the antimicrobial activity of aqueous extracts of seed of *Prosopis africana* on the test organisms showed that no diameter of zone of inhibition of was produced on the test organisms at 50 mg/ml and 100 mg/ml (0.00 mm and 0.00 mm respectively). Result also revealed that, the diameter of zone of inhibition increases with increase in concentration of the extracts with all the extracts exhibiting higher diameter of zone of inhibition at 250 mg/ml concentration (15.50 mm, 15.00 mm and 16.00 mm for aqueous, ethanol and methanol extracts respectively (Table 3).

Table 3. Antimicrobial activity of aqueous seed extracts of *P. africana* against test organisms

Test Organism	Average diameters of zone of inhibition (mm)						
	50 (mg/ml)	100 (mg/ml)	150 (mg/ml)	200 (mg/ml)	250 (mg/ml)	+ve control	-ve control
Klebsiella pneumoniae	-	-	-	13.50	15.50	18.00	0.00
Pseudomonas aeruginosa	-	-	12.50	14.00	15.00	22.00	0.00
Microsporum canis	-	-	13.00	14.50	16.00	19.00	0.00

KEY: - = No activity, +ve = Positive, -ve = Negative. All values are means of three (3) readings.

Result of the antimicrobial activity of ethanol extracts of seed of *Prosopis africana* on the test organisms showed that the test organisms were not inhibited at 50 mg/ml (0.00 mm) while, higher diameter of zones of inhibition were produced on the test organisms at 250 mg/ml (i.e. *Klebsiella pneumonia* 14.00 mm, *P.aeruginosa* 15.70 mm and *M. canis* 16.60 mm respectively) (Table 4).

Table 4. Antimicrobial activity of ethanol seed extracts of *P. africana* against test organisms

Test Organism	Average diameters of zone of inhibition (mm)						
	50 (mg/ml)	100 (mg/ml)	150 (mg/ml)	200 (mg/ml)	250 (mg/ml)	+ve control	-ve control
Klebsiella pneumoniae	-	-	13.00	14.50	16.00	18.00	0.00
Pseudomonas aeruginosa	6.00	8.00	12.60	14.00	15.00	22.00	0.00
Microsporum canis	7.00	9.00	12.50	13.60	16.50	19.00	0.00

KEY: - = No activity, +ve = Positive, -ve = Negative. All values are means of three (3) readings.

Result of antimicrobial activity of methanol extracts of seed of *P. africana* showed that no diameter of zone of inhibition was produced on all the test organisms at concentrations of 50 and 100 mg/ml. All the extracts

produced higher diameter of zones of inhibition at 250 mg/ml (15.70 mm, 15.50 mm and 16.00 mm on *K. pneumoniae*, *Pseudomonas aeruginosa* and *M.canis* respectively) (Table 5).

Table 5. Antimicrobial activity of methanol seed extract of *P. africana* against test organism

Test Organism	Average diameters of zone of inhibition (mm)						
	50 (mg/ml)	100 (mg/ml)	150 (mg/ml)	200 (mg/ml)	250 (mg/ml)	+ve control	-ve control
Klebsiella pneumoniae	-	-	13.00	14.50	16.00	18.00	0.00
Pseudomonas aeruginosa	6.00	8.00	12.60	14.00	15.00	22.00	0.00
Microsporum canis	7.00	9.00	12.50	13.60	16.50	19.00	0.00

KEY: - = No activity, +ve = Positive, -ve = Negative.
All values are means of three (3) readings.

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Result of the minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) of aqueous extracts of seed of *P. africana* on the test organisms showed that the MIC on the test organisms ranges between 150 mg/ml-200 mg/ml, the MBC for the bacterial isolates stood at 250mg/ml while MFC on the only fungal isolate was 200mg/ml. (Table 6).

Table 6. Minimum Inhibitory, Bactericidal and Fungicidal Concentrations of aqueous seed extracts on test organisms

Test Organism	MIC	MBC	MFC
Klebsiella pneumoniae	200	50	NA
Pseudomonas aeruginosa	200	250	NA
Microsporum canis	150	NA	200

Key: MIC = Minimum Inhibitory Concentration, MBC= Minimum Bacterial Concentration, MFC= Minimum Fungicidal Concentration, NA = Not Applicable.

Result of the minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC) of ethanol seed extracts showed that the MIC for all the test organisms was at100 mg/ml - 200 mg/ml while the MBC for the two bacterial isolates was 250 mg/ml and MFC was 200 mg/ml (Table 7).

Table 7.: Minimum inhibitory Concentration (MIC), Minimum Bactericidal (MBC), and Fungicidal Concentration (MFC) of ethanol seed extracts on test organisms

Test Organism	MIC	MBC	MFC
Klebsiella pneumoniae	200	250	NA
Pseudomonas aeruginosa	150	250	NA
Microsporum canis	150	NA	200

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MFC = Minimumum Fungicidal Concentration, NA = Not Applicable.

Result of the minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) and minimal fungicidal concentration (MFC) of methanol seed extract of *P. africana* on the test organisms showed that the MIC for the test organisms was between 100mg/ml-200mg/ml while the MBC was250 mg/ml for both *K. pneumoniae* and *P.aeruginosa* and MFC was200mg/ml for *M. canis* (Table 8)

Table 8. Minimum inhibitory, Bactericidal and Fungicidal Concentration of Methanol seed extract on test organisms

Test Organism	MIC	MBC	MFC
Klebsiella pneumoniae	150	250	NA
Pseudomonas aeruginosa	200	250	NA
Microsporum canis	150	NA	250

Key: MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration, MFC= Minimum Fungicidal Concentration, NA=Not applicable.

DISCUSSION AND CONCLUSION

It is well known that infectious diseases account for high proportion of health problems especially in developing countries. There is an increasing trend in the emergence of resistance to antimicrobial agents which does not only result from poor quality drugs manufactured, patient non-compliance and irrational use of antimicrobial agents, but also due to spontaneous mutations within the microbial populations (Nester *et al.,* 2002; Denyer *et al.,* 2004). This has forced scientists to search for new antimicrobial substances from various sources such as medicinal plants (Olajuyige *et al.,* 2011) including *Prosopis africana*.

The phytochemical analysis of seed of *Prosopis africana* showed the presence of at least five phytochemical constituents with aqueous seed extracts indicating the presence of saponins, tannins, terpenoids, flavonoids, alkaloids, steroids and phenols; Ethanol extracts showing the presence of saponins, flavonoids, alkaloids, glycosides and phenols while the methanol extracts showed the presence of saponins, tannins, terpenoids, flavonoids, alkaloids, glycosides and phenols. Ajiboye *et al.* (2013) had earlier reported the presence of saponins, tannins, steroids, glycosides, alkaloids in pod of *Prosopis africana* but no flavonoids. The absence could be due to the method of extraction or the mineral composition of the soil from which the plant samples were obtained. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals or phyto-constituents and are responsible for protecting the plant against microbial infection or infestations by pests (Abo *et al.,* 1991; Liu, 2004; Nweze *et al.,* 2004; *et al.,* 2009).

The quantitative analysis of seed showed high amount of alkaloids. This agrees with the work of (2012) who reported that alkaloids exist in large proportion in seed and roots naturally. The author also reported that most alkaloids are readily soluble in alcohol and sparingly soluble in water (, 2012), which could be the reason while the two alcohols used (ethanol and methanol) had higher proportion of alkaloids than aqueous extracts (Table 1.2).

The antimicrobial activity of aqueous extract of seed of *Prosopis africana* (Table 4.3) showed that the three test organisms were resistant to all the three solvent extract at 50 and 100mg/ml which is in variance with the work of Ajiboye *et al.* (2013), Eze *et al*. (2010) and Aworinde *et al.* (2016) who in their separate works reported the inhibitory effect of *Prosopis africana* on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at concentrations lower than 50mg/ml. The zone of inhibition produced by aqueous seed extract was less; it disagrees with Aworrinde *et al.* (2016) who reported no zone of inhibition on a fungal strain, *C. albicans,* though a different strain was used.

Result of the study revealed that *K.pneumoniae, M. canis* were resistant to ethanol seed extract at 50, 100 and 150mg/ml and exhibited diameters of zone of inhibition of less than 12.00 mm. This does not mean that the extracts do not possess activity. CLSI (2006) provide a baseline of activity of plant extracts to be significant above or equal to 12.00 mm. Eze *et al.* (2010) had earlier reported higher activity of ethanol extracts on both clinical and sewage isolates of *K. pneumoniae* and *P.aeruginosa*.

The antimicrobial activity of methanol seed extract on the test organisms showed that the organisms were resistant at 50 mg/ml and 100 mg/ml. This disagrees with the work of Odozi *et al.* (2014) who reported good

antimicrobial effect of methanol extract of leaf of *P. africana on Klebsiella pneumoniae* and other microorganisms more than the aqueous leaf extract of the same plant on *K. pneumoniae* and other organisms. The variance observed could be due to the difference in plant parts and concentrations used in this work. Result showed that the alcohol extracts (ethanol and methanol) had a better activity than the aqueous extracts. This agrees with works by Osho *et al.* (2011), Odozi *et al.* (2014) and Ogbeba *et al.* (2017) who separately reported a better activity of alcohol extracts of *P. africana* on some selected organisms including *K. pneumoniae,* and *P.aeruginosa* used in this research work. Also Sen *et al*. (2012) in their work on *Melia azedarach* reported a better activity of alcohol extracts than aqueous extracts. This phenomenon is due to the fact that antibiotic compounds found in plants which are aromatic or saturated molecules easily solubilized in organic solvents (Cowan, 1999).

The presence of some of these plant secondary metabolites in a significant amount in the investigated parts of *P. africana* may have conferred the antimicrobial activity on both seed extracts of the plant (Ajiboye *et al.,* 2013). In this regards, the presence of these phytochemical in the extracts may have been responsible for inhibition exhibited by both seed and extracts. Alkaloids generally present in medicinal plants play some metabolic role and control development in living system. They are also involved in protective function of animals and are used as medicine especially the steroidal alkaloids (Jeyakumar *et al.,* 2013). Alkaloids are widely well known to have antidiabetic (Sharma, 2012) and antimicrobial (Gawali and Jadhav, 2011) activities. Plants with alkaloids may have hypoglycemic effect via mechanism of insulin releasing and insulin-mimicking activity and thus improves postprandial hyperglycemia (Patel and Mishra, 2011) and antimicrobial effects due to the action of intercalates into cell wall and DNA of organisms, inhibits release of autacoids and prostaglandins, possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on central nervous system (CNS) causing paralysis (Tiwari *et al.,* 2011). Natural antioxidants mainly come from plants in the form of phenolic compounds, such as flavonoids, phenolic acids, tocopherols etc. The presence of flavonoids has important effects in plant biochemistry and physiology as antioxidants, enzyme inhibitors, precursors of toxic substances (Prabhu and Guruvayoorappan, 2012) and they are recognized to possess antidiabetic (Tanko *et al.,* 2008; Moses *et al.,* 2012; Sharma,2012) antioxidant (Lima *et al.,* 2010; Shanmugapriya *et al.,* 2012; Wadood *et al.,* 2013), antimicrobial (Lima *et al.,* 2010; Mungole *et al.,* 2010; Gawali and Jadhav,2011; Nurdiani *et al.,* 2012;)fungicidal, natural antihistaminic (Lima *et al.,* 2010; Prabhu and Guruvayoorappan, 2012), anti-inflammatory, anti-allergic and anti-carcinogenic activities (Prabhu and Guruvayoorappan, 2012).

The anti-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation (Shahriar *et al.,* 2013). Flavonoids may exert beneficial effects as antimicrobial, anti-diarrhea, anti-helminthic, anti-diabetic and anticancer by complexing with cell walls, binding to adhesions, inhibiting release of autacoids and prostaglandins, inhibiting contractions caused by spasmogens, stimulating normalization of the deranged water transport across the mucosal cells, and inhibiting gastro-intestinal release of acetylcholine (Tiwari *et al.,* 2011) and (i) enhances insulin secretion and reduces apoptosis and promotes proliferation of pancreatic β-cells; (ii) improving hyperglycemia through regulation of glucose metabolism in hepatocytes; (iii) reducing insulin resistance, inflammation and oxidative stress in muscle and fat and (iv) increasing glucose uptake in skeletal muscle and white adipose tissue (Babu *et al.,* 2013), insulin triggering and/or insulin-like properties (Ahmad *et al.,* 2000), cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behaviour respectively (Kanadaswami *et al.,* 2005).

Tannin and steroids present in the medicinal plants are responsible for anti-inflammatory (Mungole *et al.,* 2010), antibacterial (Mungole *et al.,* 2010; Gawali and Jadhav, 2011; Shanmugapriya *et al.,* 2012; Namkeleja *et al.,* 2014), antioxidant activities (Brown and Rice-Evans, 1998; Mungole *et al.,* 2010). These compounds such

as Saponin, tannins, steroids, flavonoids and alkaloids are known to have antibacterial activity against pathogens and could be used traditionally for therapeutic purposes (Usman and Osuji, 2007). The activity of the plant extracts is concentration dependent this implies that activity is pronounced at higher concentrations and vice versa (Aworinde *et al.,* 2017).

The antimicrobial activity of the plant extracts in terms of diameter of zones of inhibition produced on the test organisms though was considered good, was less than the zones of diameter of inhibition produced by the positive controls (ampicillin for the bacterial isolates and Griseofulvin on the fungal isolate). This agrees with the work of Ajiboye *et al.* (2013) who reported that the positive control (ciprofloxacin) produced a better inhibitory effect than the plant extracts. This could be due to the fact that the plant extracts are not purified, whereas the positive control is well purified.

Result for MIC, MBC and MFC showed that MICs where at lower concentrations while the MBCs and MFCs where at higher concentrations. This result agrees with Sen*et al*. (2012) who reported a similar phenomenon in their work, suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentrations (Thatoi *et al.,* 2008). The result obtained in this work justifies the use of *P, africana* traditionally as a medication for treating certain ailments and proved that *P, africana* can be a source for a new antibiotic if well harnessed.

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