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PHYTOCHEMICAL ANALYSIS OF CRUDE METHANOL EXTRACTS AND ANTIMICROBIAL ACTIVITY OF N-HEXANE FRACTIONS OF METHANOL SEED AND POD EXTRACTS OF PROSOPIS AFRICANA ON SOME SELECTED MICROOGANISMS

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ABSTRACT
Antimicrobial activity of n-hexane fraction of methanol seed and pod extracts of *Prosopis africana* on clinical isolates of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Microsporum canis* was determined using agar well diffusion method. Cold maceration using methanol for extraction and gas chromatography mass spectrometry (GC-MS) was for determination of the active phytocosntituents. Qualitative analysis showed the presence of at least six or more of tannins, flavonoids, alkaloids, phenols, glycosides, steroids, saponins and terpenoid. The quantitative analysis showed that the extracts were higher in tannin and alkaloids (1300 mg/100 g, 23.40 % and 810 mg/100g, 20.0 %) for seed and pod extracts respectively. All the extracts showed antimicrobial activity against the tested organisms with average diameter of zone of inhibition ranging between 10.00 -15.60 mm. Result of the GC-MS showed the presence of at least two or more of n-Hexadecanoic acid, oleic acid, stearic acid (Octadecanoic acid), hexadecanoic acid, 9-Octadecenal, Hexadecanioc acid 2,3-dihydroxylpropyl ester and other various biological components with various biological activities. The results obtained in this work showed that *Prosopis africana* can be useful in treating ailments caused by pathogenic organisms selected in this work.

Keywords: *antimicrobial activity, phytochemical constituents, gas chromatography mass spectrometry.*

INTRODUCTION

Medicinal plants are a group of species that accumulate different active principles, useful in treating various human or animal diseases. The long use of herbs in medicine is a sure indication of their value and usefulness in the future. In modern medicine, the importance of medicinal plants is increasing (1) with pharmaceutical and cosmetic industries increasingly using plant resources from rural or polluted areas.

Among the successes, achieved by herbal medicine is that many drugs in clinical use today were discovered from the way plants were used in traditional communities. Examples include quinine which was discovered from the way traditional communities in South America especially Peru, Columbia and Bolivia, used plant species of the genus *Cinchona* in managing fevers. Digitoxin is a popular heart tonic and was obtained from *Digitatis pupurea*, a plant that was used as a tonic in traditional communities in Europe. Toxol is a modern therapy for ovarian cancer obtained from *Taxus brerifolia* which was a traditional medicine plant in British Columbia (2, 3).

Plants base natural constituents can be derived from barks, leaves, flowers, roots, fruits, seeds of plants (4) which in most cases contain active components (5).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 (6), anti-inflammatory, antiviral and cytotoxic activities (7). However, the bioactive constituents conferring these properties on many plant species also been implicated in allelopathy (8). Allelopathy has been defined as the effect (s) of one plant on other plants through the release of chemical compounds in the environment (9).

Studies have given credence to the folkloric claims on the prophylactic and therapeutic effectiveness of some medicinal plants against infectious agents including multidrug resistant ones (10 - 13). Notable among them is *Prosopis africana* (14).

The *Prosopis africana* tree is common in the Middle Belt and Northern parts of Nigeria and are refered to as "Kiriya" and "Okpehe" in Hausa and Idoma languages (15) 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 (15).

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 (16), pods are used for treating wounds (17). The purpose of this work is to evaluate the phytochemical analysis of crude methanol extracts of *P. africana* and determine the antimicrobial activity of n-hexanoic fractions of seed and pod of *P. africana* on some selected microorganisms.

METHODS

Collection and Identification of Plant Sample

Dried pods (Plate 1a) containing seeds (Plate 1b) 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.

Plant Processing and Solvent Extraction

The pods containing seeds were broken and the seeds removed and separated from the pod and both dried under shade until constant weight for 7 days. The seeds and pods were pounded separately using mortar and pestle into powdered form. Five

hundred (500) grams powdered material was weighed and soaked in 1.5 L 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 extracts were then divided into two portions, the first portion of each solvent extract was used for phytochemical screening (qualitative and quantitative), the second portions were reconstituted in 500 ml 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 $^{\circ}$C until when needed for susceptibility testing.

Phytochemical Analysis

Qualitative Analysis

The qualitative phytochemical analysis of pod of *P. africana* was carried out using the standard procedures described elsewhere (18,19).

Test for Saponins (Froting test)

To 5 ml 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 (18)

Test for Tannins

To 1 ml 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 (18).

Test for Terpenoids

To 2.0 ml of each solvent extract in a tube, 2.0 ml of chloroform was added the 3 ml 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 (18).

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 (18).

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 (19).

Test for Glycosides

To 2.0 ml of each solvent extracts, 1 ml 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 (18).

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 (18).

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 (18).

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 $^{\circ}$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 $^{\circ}$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 (18). 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, 2.5 ml of standard tannic acid solution was dispensed into a separate 50.0 ml flask. The absorbance was measured at 250 nm and compared to the standard tannic curve obtained by plotting optical density (absorbance) versus tannic acid concentration (20). The following formula was used in the calculation:

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

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 h. The extract was filtered and the filtrate extracted with petroleum ether (60 – 80 °C) and concentrated to dryness. The dried ether extraction was treated as total terpenoids (21). 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 (10 g) 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 (22). 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 (5 g) of each sample was weighed into a 250 ml beaker and 200 ml of 100 % acetic acid in ethanol was added and covered and allowed to stand for 4 h. 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 (19). 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 was carried out as described elsewhere (23). 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:

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

Steroids Determination

This was determined according to the method of Harborne (18). 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 °C 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 (Spectrophotometric Method)

One gram (1 g) of the fat free sample was boiled with 50 ml of ether for the extraction of phenolic component for 15 min. 5ml of the extract was taken into 50ml flask distilled water was added. 2 ml 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 (24). These were measured at 505 nm. These data were used to estimate the total phenolic content using a standard calibration of gallic acid.

Test for Purity of Extracts

Both plant extracts fractions were plated on the nutrient agar and incubated at 37 °C for 24h 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 before being used for the antimicrobial activity.

Determination of antibacterial activity of extracts

The antimicrobial activity of the *n*-hexane fractions of both seed and pod of *Prosopis afriacana* was carried out on the test organisms using agar well diffusion method method (25). Mueller Hinton agar (MHA) was seeded with test bacteria adjusted to 0.5 McFarland turbidity standard according to CLSI (26) using sterile swab stick. Wells (6 mm diameter and 2 cm 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, 100, 150, 200 and 250 mg/ml). Aliquote of 0.1 ml (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 °C for 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.

Determination of antifungal activity of extracts

The antifungal activity was carried out using the pour plate method (27). 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 Sauboraud's Dextrose Agar (SDA) previously cooled to 50 °C and which had been supplemented with chloramphenicol and cyclohexamide to prevent bacterial growth. Wells (6 mm and 2 cm apart) were made using sterile cork borer and 100 µl (0.1 ml) of each fraction of extracts was transferred into the wells using sterile pipette and incubated at 28 °C for 4 days. Griseofulvin (10 mg) and distilled water was used as positive and negative control respectively. The diameter of zone of inhibition (mm) around the wells was measured in three different directions and average taken using ruler.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

This was carried out on a GC-MS-QP 2010 plus Shimadzu system and Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused capillary column (30 m x 0.25 1D µl df, composed of 100 % dimethyl polysiloxane). For GC-MS detection, an election ionization system with ionization energy of 70ev was used. Helium gas (99.99 %) was used as the carrier gas at constant flow rate 1.0 ml/1 min and an injection volume of 2ul was employed (split ratio of 10:1) injector temperature – 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10 °C/ min to 200 °C then 5 °C/min to 200 °C then 5 °C/min to 280 °C/min, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 40 to 550 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed the NIST Ver. 2.0 year 2009 library.

Interpretation on mass spectrum of GC-MS was done using the data base of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the test materials were ascertained.

RESULTS

Result of the phytochemical analysis of various solvents (Aqueous, Ethanol and Methanol) of seed and pod of *Prosopis africana* showed the presence of saponin, flavonoids, alkaloids and phenols in all the solvents extracts of seed and pod (Table 1). Result also showed that tannin, terpenoids and steroids were absent in the ethanol seed extracts, steroids were absent in the methanol seed extracts and glycosides were absent in the aqueous seed extracts. Result also revealed that while the ethanol pod extracts contains all the phyto-constituents tested, the methanol pod extracts lacked glycosides and steroids while the aqueous pod extracts lacked only glycosides.

Quantitative analysis of seed and pod of *Prosopis africana* showed that methanol seed extracts was higher in tannins (1300 mg/100g), methanol pod extract was higher in alkaloids (20.00 %, Table 2). The antimicrobial activity of n-hexane fraction of methanolic seed extract of *P. africana* on the test organisms showed that the bacterial isolates were resistant at 50 and 100 mg/ml of the extract while the fungal isolate was resistant at 50 mg/ml. The table also revealed that highest diameter zones of inhibitions were produced at 250 mg/ml on all the test microorganisms (15.00, 14.00 and 15.60) on *K. pneumoniae, P. aeruginosa* and *M. canis* respectively (Table 3).

The antimicrobial activity of n-hexane fraction of methanolic pod extract of *P. africana* on the test organisms revealed that the organisms were resistant to the extracts at 50 and 100 mg/ml with highest diameter zones of inhibition produced at 250 mg/ml(14.30, 14.70 and 15.40) on *K. pneumoniae, P. aeruginosa* and *M. canis* respectively (Table 4).

The results of the gas chromatography mass spectrometry (GC-MS) of methanol seed extract (Table 1.5 and Figure 1) showed thirteen components. These are: n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, 9-Tetradecenal, oleic acid, Octadecanoic acid, Octadecanoic acid ethyl ester, Palmitoyl chloride, undecenoic acid, 9-Octadecenal, 1-Octanol,2-butyl, Hexadecanoic acid, 2,3-dihydroxypropyl ester, 7,11-Hexdecadienal, and 9,l7-octadecadienal. The result also showed that oleic acid had had the highest peak area (31.58 %) while 1-Octanol, 2-butyl has the lowest peak area (1.74 %) (Table 5).

GC-MS results of Methanol Pod extract (Table 6 and Figure 2) showed 15 chemical compounds and revealed that oleic acid was the highest, followed by Hexadecanoic acid, stearic acid was next while (E) 13 Docosenoic acid was least.

DISCUSSION

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 (28, 29). This has forced scientists to search for new antimicrobial substances from various sources such as medicinal plants (30) including *P. africana*.

The phytochemical analysis of seed and pod of *Prosopis africana* showed the presence of at least six phytochemical constituents. Ajiboye *et al.* (31) 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 (32 - 35).

The quantitative analysis of seed and pod of *P. africana* showed high amount of alkaloids in seed than in pod solvent extracts. This agrees with the work of Doughari (36) 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 (36), 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 *n*-hexane fraction of methanolic 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.* (37) 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.

The antimicrobial activity of *n*-hexane fraction of methanol pod extracts revealed that all the organisms were resistant at 50 and 100 mg/ml. The non-activity of the extracts at 50 mg/ml used in this research work contrasts results of other findings (31, 38) who reported good activity of *Prosopis africana* on some microorganisms at concentrations lower than 50 mg/ml. This could be because the patients from whom the organisms were obtained were on antibiotic medication. The activity of methanol extracts obtained in this work agrees with the work of Odozi *et al.* (37) who reported a good activity of *Prosopis africana* on some selected microorganisms, although Odozi and his group of researchers used higher concentrations than the ones used in this present work.

The presence of some of these plant secondary metabolites in a significant amount in the investigated part of *P. africana* may have conferred the antimicrobial activity on both seed and pod extracts of the plant (31). In this regards, the presence of these phytochemical in the extracts may have been responsible for inhibition exhibited by the pod extracts.

Alkaloids are widely well known to have antidiabetic (39) and antimicrobial (40) activities. The presence of flavonoids has important effects in plant biochemistry and physiology as antioxidants, enzyme inhibitors, precursors of toxic substances (41) and they are recognized to possess antidiabetic (39, 42, 43) antioxidant (44-46), antimicrobial (44, 47-49) fungicidal, natural antihistaminic (41, 44), anti-inflammatory, anti-allergic and anti-carcinogenic activities (41). Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds (49-52) and flavonoids (43-45). Phenols are phytochemical compounds that function in nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy in herbs. The phenolic compounds, have biological and pharmacological properties especially

their antimicrobial activity (47, 52), antiviral, anti-inflammatory, cytotoxic activity, anti-mutagenic and anti-carcinogenic activities (42).

Terpenoids also exhibit antimicrobial and anti-diarrhoeal effects by membrane disruption and inhibits release of autacoids and prostaglandins (53). Saponins have been reported to possess antidiarrheal (42) piscicidal and for controlling cholesterols (53, 54) and anticancer. Anti-diarrhoeal, anticancer and anthelmintic activities may be due to inhibits histamine release in vitro, possesses membrane permeabilising properties and leads to vacuolization and disintegration of teguments respectively (53), saponin is also reported to be an important expectorant, effective for the treatment of respiratory infections (55) and its presence in the plant parts used in this studies justifies why the plant *P.africana* is used traditionally for the treatment of respiratory tract infections. Tannin and steroids present in the medicinal plants are responsible for anti-inflammatory (46), antibacterial (44, 46, 47, 52), antioxidant activities (46, 49).

Glycosides have been reported have antidiarrhoeal effect may be due to the action of inhibits the release of autacoids and prostaglandins (53). Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones; they also promote immune functions in the skin and also reduce inflammation (56-58). A large number of therapeutic agents in use today have been isolated or derived from plant sources (59).

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 (59). The activity of the plant extracts is concentration dependent this implies that activity is pronounced at higher concentrations and vice versa (21).

Result of Gas Chromatography Mass Spectrometry (Tables 1 and 2, and Figures 1 and 2) showed the various components which were identified using NIST library by comparing the retention time, molecular formula, and peak area. All the solvents extracts contains at least two or more of *n*-Hexadecanoic acid, oleic acid, stearic acid, hexadecanoic acid (palmitic acid), 9,17-Octadecadienal. *n*-hexadecanoic acid have been reported to have antimicrobial activity (59-63), and antifungal activity (64). Oleic acid is reported to have antimicrobial effect (65). Choi *et al.* (66) reported that antimicrobial activity of long chain unsaturated fatty acids have been well known for years. Long chain unsaturated fatty acids such as linoleic and oleic acids are bactericidal to important pathogenic organisms including methicillin-resistant *Staphylococccus aureus, Helicobater pylori* and *Candida albicans.* Oleic acid has been found to inhibit bacterial enoyl-acyl carrier protein reductase (Fab1), an essential component of bacterial fatty acid synthesis which has served as a promising target for antimicrobial drugs (67). Stearic acid, a saturated fatty acid is reported to have antimicrobial effects (64). Duke (63) reported Hexadecanoic acid to also have antidiarrheal activity. Hexadecanoic acid,2,3-dihydroxypropyl ester present in ethanol pod extract is reported to have antimicrobial activity and anticancer activity (68). 9-Octadecenal found in the extracts is reported to have antimicrobial activity (69) and anti-inflammatory (69), 5α reductase inhibitor, percutanea stimulant and anemiagenic (70). Although it was not in large quantity in some solvent extracts. 1-Octadecyne is reported to have anti-inflammatory, antibacterial and fragrance activity (71). 9,17-Octadecadienal found in (Table 1.1 and 1.2) is reported to have anti-inflammatory and antioxidant activity, antimicrobial activity (72).

Conclusion

The presence of some of these plant secondary metabolites in a significant amount in the investigated parts of *P. africana* was responsible for the exhibition of antimicrobial activity by both the seed and pod extracts. In this regards, the presence of these phytochemical in the extracts may have been responsible for inhibition exhibited by the pod extracts. Further identification of these active chemical components using Gas Chromatography Mass Spectrometry provide potential for synthetic development of novel antimicrobials using the identified components. Demonstration of antimicrobial activity against the range of organisms tested showed that *P. africana* can be useful in

treating ailments caused by these selected pathogenic organisms.

DECLARATIONS

Ethics Approval and Consent to Participate

Ethics approval and consent to participate no. ADHM/EC/2019/R00815 was obtained from the Ethics Committee, Adamawa State Ministry of Health, Yola, Nigeria

Consent for Publication

The manuscript does not contain any individual person's data in any form (including individual details, images or videos) and no presentation of case reports was involved.

Availability of Data and Material

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Competing Interests

Prof. James Hamuel Doughari, and Mom Saa-Aondo declare that they have no conflict of interest.

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Authors' Contributions

Prof. James Hamuel Doughari conceptualized, designed, reviewed, edited and approved the draft manuscript.
Mr. Mom Saa-Aondo collected and analyzed the data and drafted the manuscript.

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Plate 1: a) Pods and b) Seeds of *Prosopis africana* (Source: Field work, 2019)

Table 1. Phyto-constituents of seed and pod of *Prosopis africana*

Phyto-constituents	Meth (p)	Meth (s)
Saponin	+	+
Tannin	+	+
Terpenoid	+	+
Flavonoids	+	+
Alkaloids	+	+
Glycosides	-	+
Steroides	-	-
Phenols	+	+

Key: + = Present, - = Absent, Meth=Methanol, P= Pod, S=Seed.

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

Concentration of Phyto-constituents	Composition of components in extracts	
	Meth (p)	Meth (s)
Saponin (%)	18.20	13.60
Tannin (mg/100g)	1300	810
Terpenoid (%)	4.00	6.50
Flavonoids (%)	4.90	7.90
Alkaloids (%)	23.40	20.00
Glycosides (mg/100g)	140	-
Steroides (%)	-	-
Phenols (mg/100g)	0.94	0.55

Key: - = Absent

Table 3. Antimicrobial activity of n-Hexane fraction of methanol seed extracts of *P.africana* on test organisms

Test Organisms	Average Diameter of Zone of Inhibition (mm)				
	50 (mg/ml)	100 (mg/ml)	150 (mg/ml)	200 (mg/ml)	250 (mg/ml)
K. pneumonia	-	-	12.00	14.00	15.00
P.aeruginosa	-	-	12.00	13.60	14.00
Microsporum canis	-	10.00	12.30	13.00	15.60

Key: - = No activity

Table 4. Antimicrobial activity of n-Hexane fraction of methanol pod extract of *P.africana* on test organisms

Test Organisms	Average Diameter of Zone of Inhibition (mm)				
	50 (mg/ml)	100 (mg/ml)	150 (mg/ml)	200 (mg/ml)	250 (mg/ml)
K. pneumonia	-	-	12.00	13.00	14.30
P.aeruginosa	-	-	12.00	13.50	14.70
Microsporum canis	-	-	12.80	14.00	15.40

Key: - = No activity.

Table 5. Compounds detected in methanol seed extracts of *P. africana.*

S/No	Constituents	Nature of Compound	Area (%)	RT (min)
1.	n-Hexadecanoic acid		7.83	15.99
2.	Hexadecanoic acid, ethyl ester		3.65	16.11
3.	9-Tetradecenal		2.72	17.97
4.	Oleic acid	Flavonoids	31.58	17.73
5.	Octadecanoic acid		5.68	17.88
6.	Octadecanoic acid ethyl ester		2.73	19.98
7.	Palmitoyl Chloride		2.26	18.91
8.	Undecenoic acid		12.60	19.44
9.	9-Octadecenal		7.44	20.41
10.	1-Octanol, 2-butyl		1.72	20.59
11.	Hexadecanoic acid, 2, 3	Ester	4.08	20.78
12	dihydroxypropyl ester		4.55	22.09
13	9,12-Hexadecadienal		13.18	22.37
	9,17-Octadecadienal			

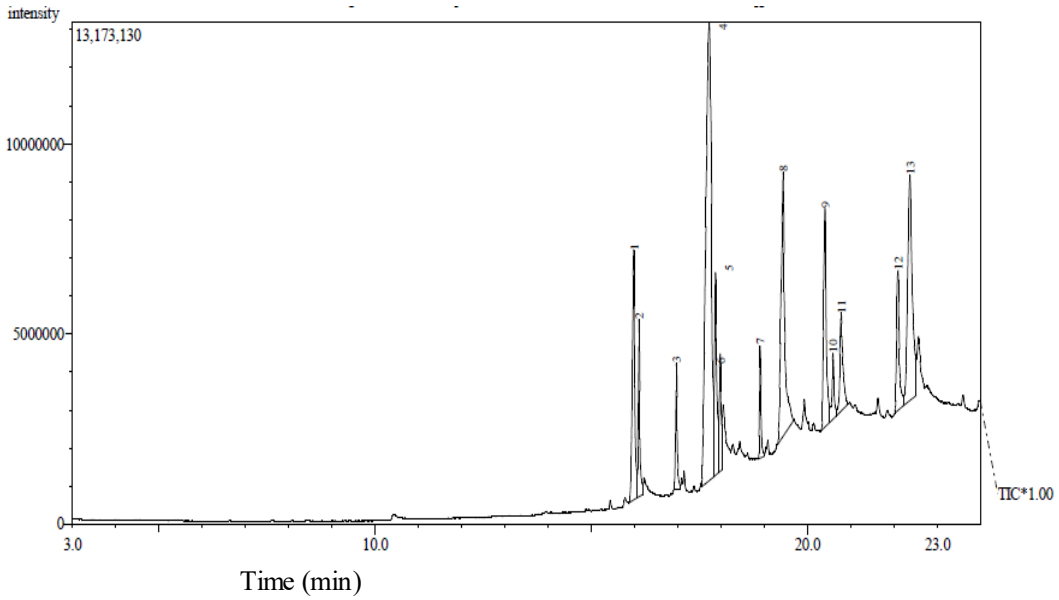

Figure 1. Gas Chromatogram of methanol seed extracts of *P. africana*

Table 6. Compounds detected in methanol pod extracts of *Prosopis africana*

Peaks	Constituents	Nature of compound	Area (%)	RT (min)
1	E)13-Docosenoic acid		0.53	10.449
2	1-Otadecyne		3.67	15.288
3	2-Heptadecanone		2.47	15.382
4	(7Z)-Hexadecenal		1.17	16.057
5	1-Hexadecyne		1.23	16.858
6	n-Hexadecanoic acid		12.10	17.974
7	11-Octadecenoic acid, methyl ester		1.79	19.984
8	Pentadecanoic acid		1.50	20.227
9	Oleic acid	Flavonoid	51.55	20.905
10	Stearic acid		11.98	21.122
11	Hexadecanioc acid 2,3-dihydroxylpropyl ester		1.12	22.456
12	E-2-Octadecadecen-1-ol		1.43	22.728
13	n-Hexadecanoic acid		4.19	23.306
14	9-Octadecenal		2.60	24.275
15	Hexadecanal		2.69	24.782

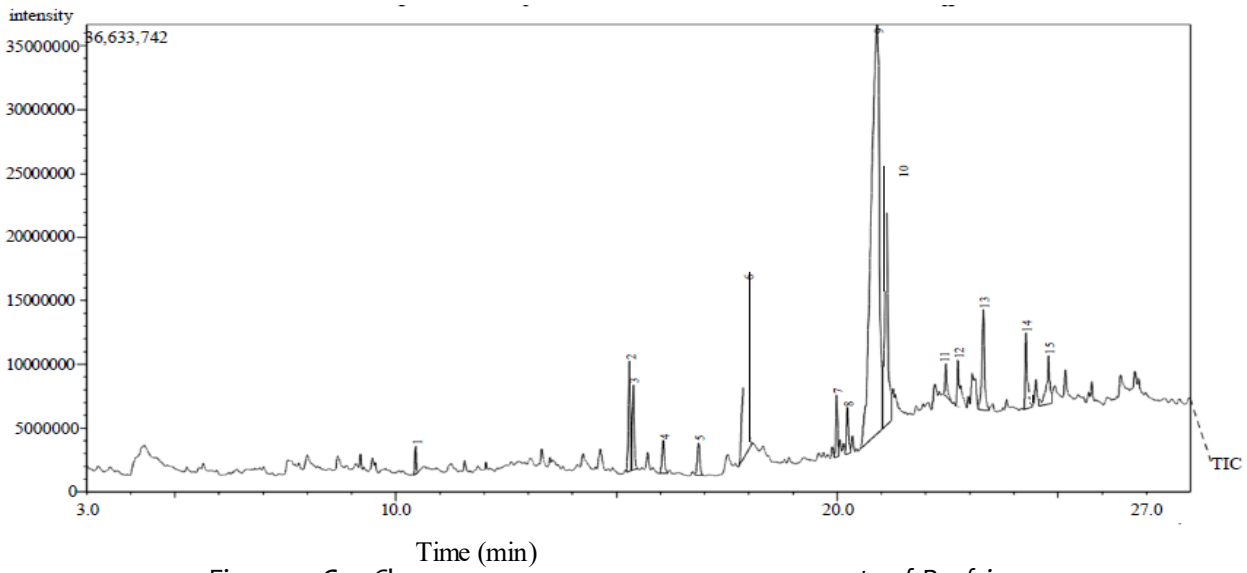

Figure 2. Gas Chromatogram of methanol pod extracts of *P. africana*