



ANTIBACTERIAL, PHYTOCHEMICAL AND PROXIMATE ANALYSIS OF *PROSOPIS AFRICANA* (LINN) SEED AND POD EXTRACT

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ABSTRACT

This study was carried out to determine the antibacterial activities of seed and pod extracts of *Prosopis africana* at 25mg/ml concentration against fifteen bacterial isolates namely: *Klebsiella pneumoniae*, *Bacillus anthracis*, *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus polymyxa*, Methilin-resistant *Staphylococcus aureus* (MRSA), *Proteus vulgaris*, *Corynebacterium pyogenes*, *Micrococcus leutus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescence*, *Streptococcus fecalis* and *Escherichia coli*. The results obtained showed that all the isolates appeared to be susceptible to Ciprofloxacin (control) with the zone of inhibition ranging from 25mm-50mm. The pod extract was susceptible to *K. pneumoniae*, *B. anthracis*, *B. stearothermophilus*, *B. polymyxa*, *Clostridium pyogenes*, *Pseudomonas aeruginosa*, *B. cereus*, *Staphylococcus aureus*, *P. fluorescence*, *Streptococcus fecalis*, *E. coli* were having zones of inhibition to be between 8mm-17mm in diameter. For the seed extract, *K. pneumoniae*, *B. polymyxa*, *E. coli*, *S. aureus*, *S. fecalis*, *P. aeruginosa*, *B. subtilis* were susceptible with the zones of inhibition ranging between 5mm-14mm. The minimum inhibitory concentration (MIC) analysed on the seed extract showed at least 1.562% in the bacterial strains tested. The phytochemical analysis of the seed extract revealed that saponin, alkaloids, steroids, flavonoids, phlabotanin and tannin were present in the extracts of seed and pod. However cardiac glucosides was absent in the seed extract. The proximate composition consists of moisture content (3.3±0.0%), total ash (4.4±0.1%), crude protein (23.6±1.5%), crude fibre (54±0.8%) and carbohydrate (1.9±0.3%). The predominant mineral found in the seed were Potassium, (K) (617.5mg/100g sample), Magnesium (Mg) (420.1mg/100g sample). Others were Sodium (Na), Calcium, Phosphorus, Manganese (Mn), Copper (Cu), Zinc (Zn), and iron (Fe) were 110.7, 362.5, 196.4, 36.2, 46.2, 22.4 and 15.5mg/100g sample respectively. This study indicates the potential efficacy of *Prosopis africana* in the treatment of various infections caused by the test organisms.

Keyword: *Prosopis africana*, Phytochemical screening and Seed

INTRODUCTION

The trees of *Prosopis africana* are common in the Middle belt and Northern parts of Nigeria and are referred to as “Kiriya” and “Okpehe” in Hausa and Idoma/Tiv languages in Nigeria respectively (Ajiboye, 2009). In many areas where the trees are grown or available, the fermented seeds of *P. africana* are used as a food condiment; its young leaves and shoot are fodder that is highly sought after towards the end of the dry season. The wood has a high

calorific value of about 1720joules/kg, thereby produces excellent charcoal and firewood. *Prosopis africana* yields a gum, tannin or dyestuff, the bark and roots contain 14-16% tannin and a colouring matter that gives a reddish tint to leather, pounded dry fruits are suitable as a fish poison (Ajiboye, 2009). *Prosopis africana* is used to control erosion, the bark is used to make beehives, the leaves and shoots are palatable to livestock and suitable for shades in homesteads in dry areas. It has the

potential to fix atmospheric nitrogen; provides useful mulch for soil improvement, suitable as an avenue tree, its pod ashes are a source of potash for soap making, it has a potential for parkland agroforestry systems and for improved agroforestry technologies (Ajiboye, 2009). *Prosopis* plantation has also been established primarily for fuel wood production with the belief that such plantings would also benefit the environment. Almost all parts of the tree is medicinal, the leaves in particular for the treatment of headache and toothaches as well as other various ailments its leaves and bark are combined to treat rheumatism, remedies for skin diseases, fever and eyewashes are obtained from the bark. The roots are a diuretic and are used to treat gonorrhea, tooth and stomach ache, dysentery and bronchitis. In Mali, the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps (Ajiboye, 2009). Dry pods are used as fish poisoning and for treating wounds and tooth decay are edible (Abbiw, 1990). The tree is planted as windbreaks, hedges, soil conservation and it improves the soil through nitrogen fixation.

It is well known that diseases caused by pathogenic microorganisms account for high proportion of health problems, especially in the developing countries (Olajuyigbe *et al.*, 2011). 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).

The essence of this work is to evaluate the antibacterial, phytochemical and proximate analysis of the seeds and pods extract against some microorganisms. Moreover, this research work would further expose individuals to different ways of eradicating or minimizing the existence of common diseases using local or alternative methods. The physiology status of the pod and seed of this plant are obscured in the literature; therefore, this work would expose

scientific researchers to obtaining the necessary information on the pods and seeds of the plant.

MATERIALS AND METHODS

Sample collection

Dried pods of *Prosopis africana* (Linn) were collected on the 7th February, 2012, from the campus of University of Ilorin, Kwara State, Nigeria. The pods were sorted to remove stones and bad pods.

Seed extraction

One hundred grammes (100g) of each plant part were ground with local grinding machine and were soaked separately in 360ml of methanol and 240ml of sterile distilled water (ratio 4 : 2) for four days at 30 – 32^oC. The extracts were filtered through a Millipore filter (0.25µm). The resulting filtrate was concentrated under reduced pressure at 50^oC and then transferred into a well labeled sterile bottle. The same concentrate was used for determination of phytochemical, mineral and proximate analysis of the pod and seed extract.

Test organisms

Pure cultures of *Escherichia coli*, *Bacillus anthracis*, *Proteus vulgaris*, *Enterococcus aerogenes*, *Klebsiella pneumoniae*, *Clostridium pyogenes*, *Bacillus stearothermophilus*, *Micrococcus leutus*, *Staphylococcus aureus*, *Staphylococcus fecalis*, *Bacillus. polymyxa* and *Bacillus subtilis*, were obtained from the Medical Microbiology Department of Obafemi Awolowo Teaching Hospital, Ile –Ife, Nigeria. Bacterial cultures were maintained on Nutrient agar slant, while antibacterial assay was carried out using Mueller Hinton agar and stored for at 37^oC for 18-24 hours.

Preparation of Bacterial Isolates

Bacterial isolates from the stock cultures were streaked onto nutrient agar plates and incubated for 18-24hr.

Antimicrobial assay

Antibacterial activity was determined by the agar-well diffusion method according to the NCCLS (NCCLS, 1993). Stock cultures of the isolates were sub cultured into a sterile nutrient broth and were incubated for 18hrs. From the 18hr broth, the organisms were inoculated into 20ml of Mueller Hinton agar in a MacCartney bottle, shaken to allow even distribution of the organisms, dispensed into petri plates and

allowed to set. Wells (6 mm diameter) were cut into the agar using a sterile cork borer and one milliliter of the plant extract and the antibiotic were tested in a concentration of 25mg/ml. Culture plates were incubated at 37°C for 24h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zones formed around the wells. A standard 25mg/ml ciprofloxacin was used as a positive control.

Phytochemical screening of the seed and pod extracts

The methanolic extracts of *Prosopis africana* were analyzed by qualitative method for the presence of alkaloids, saponins, steroids, flavonoids, phlobatannin, alkaloids and cardiac glycosides (Harborne, 2005).

Test for alkaloids, Cardiac glycosides, Flavonoids, Saponin, Steroids, Tanins, Carbohydrate and Protein

About 6 drops of Mayer's reagent, Dragendroff's reagent and 1% HCl were added to the extract. An organic precipitate indicated the presence alkaloids in the sample (Kumar *et al.*, 2009).

Cardiac glycosides test was carried out using 5ml of the extract and added to 2ml of chloroform and 3ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of conc. H₂SO₄. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might form just gradually throughout thin layer (Ayoola *et al.*, 2008).

The flavonoid test is carried out by adding 5ml of diluted ammonia solution to a portion of plant extract followed by addition of concentrated H₂SO₄. A yellow colouration indicates the presence of flavonoids. (Ayoola *et al.*, 2008).

The Saponin extract was measured and about 5ml was added to 20ml of distilled water and afterwards agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins according to Kumar *et al.* (2009).

The preparation of steroid test was carried out according to Edeoga *et al.* (2005), 2ml of acetic anhydride was added to 0.5g of the extract with 2ml of H₂SO₄. The colour change from violet

to blue or green indicated the presence of steroids.

Test for tannin required a 5ml of the extract was added to a few drops of 1% lead acetate. A yellow precipitate indicated the presence of tanins (Edeoga *et al* 2005).

Carbohydrate test required few drops of Molisch's reagent was added to 2ml of the extract and shaken well. 2ml of conc. H₂SO₄ was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Protein is tested for by adding 2ml of protein solution to 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO₄ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Mineral analysis

The mineral content were analyzed from solutions obtained by first dry-ashing the seed flour at 550°C the ash obtained was boiled with 15cm³ of 20% hydrochloric acid in a beaker, filtered into a 100cm³ standard flask and made up to the mark with distilled water. Sodium and potassium were determined by using a flame photometer, phosphorus by using colorimeter and other metals by AAS (White house *et;al.*, 1945).

Determination of moisture content

Empty foil was dried in the oven at 105°C put in the desiccators to cool weighed and s was recorded as W₀. Then 5g of the sample was weighed in the foil, put into the oven at 105°C for 2hours to dry and recorded as W₁. The sample was transferred into the desiccators after 2hours in the oven to cool and td, the value recorded as W₂ and calculated as:

$$\frac{W_1 - W_2}{W_1 - W_0} \times 100$$

Where:

W₀ = weight of empty foil.

W₁ = weight of sample before drying.

W₂ = weight of sample after drying.

The moisture content is recorded in percentage (%) (White house *et;al.*, 1945)

Determination of ash content

The total ash of substance is the inorganic residue remaining after the organic matter has been burnt away. The empty crucible was dried

in the furnace at 550°C put in the desiccators to cool and weighed, the value recorded as W_0 . 1g of sample was weighed on the crucible and the value was recorded as W_1 , the sample was put into the furnace at 550°C for 6hours or until it turns grey to burn off the organic matter leaving the inorganic matter and transferred to the desiccators to cool when the organic matter had been burned off before weighing the sample and the value was recorded as W_2 in percentage (%).

Calculation

$$\frac{W_2 - W_0}{W_1 - W_0} \times 100$$

Where; W_0 = empty crucible

W_1 = empty crucible + sample before drying.

W_2 = empty crucible + sample after drying.

(White house *et;al.*, 1945)

Determination of Crude fibre content

The sample were de-fatted with petroleum ether or hexane. A gram defatted samples was weighed into 600ml beaker and 100ml of TCA reagent was added and boiled, refluxed for 40minutes. The flask was removed and allowed to cool down and then filtered. The residue was washed for six times with hot distilled water and once with methylated spirit. The sample on filter paper was transferred into a porcelain crucible, dried in the oven at 100°C overnight. These were cooled in a dessicator and weighed termed A. The ash in a furnace at 600°C or 6hrs was allowed to cool in a desicator and also weighed. This was also termed B. Therefore, %Crude fibre = $\frac{(\text{weight A}) - \text{Weighed B}}{\text{Sample weight}} \times 100$

(White house *et;al.*, 1945).

RESULTS

All the isolates used were susceptible to the antibiotic (ciprofloxacin) used as control. The pod extract was also susceptible to *Klebsiella pneumoniae*, *Bacillus anthracis*, *Bacillus stearothermophilus*, *Bacillus polymyxa*, *Clostridium pyogenes*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Pseudomonas fluorescense* *Streptococcus fecalis* and *Escherichia coli* (Fig 1) with zones of inhibition measured in 'mm' to be 12,11,12, 14, 11, 10,10, 15,10 respectively. Fig 1 showed that *S. fecalis* had the highest susceptibility to the pod extract having 15mm recorded zone of inhibition followed by *B. polymyxa* with 14mm. *Bacillus*

cereus, *E. coli* and *P. aeruginosa* had the lowest susceptibility with zone of inhibition of 10mm while *Micrococcus leutus*, *Proteus vulgaris*, *Staphylococcus aureus* and Methilin- resistant *Staphylococcus aureus* were resistant to the pod extract. Test of seed extract against *K. pneumoniae*, *B. polymyxa*, *E. coli*, *S. aureus*, *S. fecalis*, *P. aeruginosa*, *B. subtilis* showed zones of inhibition represented as 14, 7, 7, 6, 9, 5, 9 all recorded in 'mm' respectively. *Klebsiella pneumoniae* had the highest zone of inhibition (14mm in diameter) which means that it was the most susceptible, *Streptococcus fecalis* and *B. subtilis* were next with 9mm zone of inhibition, *S. aureus* had 6mm in diameter, while *P. aeruginosa* had the least susceptibility with 5mm in diameter. *Bacillus cereus*, *C. sporogenes*, *B. stearothermophilus*, *Micrococcus leutus*, *P. fluorescense*, *B. anthracis*, *Proteus vulgaris* and Methilin-resistant *S. aureus* were resistant (Fig 2). In total, *K. pneumoniae*, *B. polymyxa*, *E. coli*, *S. fecalis* and *B. subtilis* were susceptible to the two extracts used. MIC was done on the susceptible isolates at different concentrations of 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, and 1.562mg/ml. The result showed that *B. cereus*, *Cornyobacterium pyogenes*, *P. fluorescense* and *E. coli* needed just very little concentration of about 1.562mg/ml to inhibit their growth while *B. subtilis* needed a higher concentration of about 6.25mg/ml (Fig 2). The quantitative phytochemical analysis from this study showed that the seed extract of *P. africana* contains saponins, alkaloids, phlabotannins, steroids, flavonoids and tannins but lacks cardiacglucoside (Table 1). Whereas all other test parameters were present in pod extract except flavonoids (Table 1). Table 2 showed the mineral composition of *P. africana* seed tested in this study. It was observed that potassium was the most abundant mineral this was 617.1mg/100g. Magnesium was found to be the next highest mineral component of 420.1mg/100g sample, calcium (362.5), phosphorus (196.4), sodium (110.5mg/ml), copper (46.6mg/ml), zinc (22.5mg/ml) and iron (15.5mg/ml). The seed was richer in potassium, magnesium, calcium and phosphorus but values of copper, zinc, iron and manganese were low while lead was not detected.

key to legends:CIP= ciprofloxacin, MSE= methanoic seed extract, MPE= methanoic pod extract

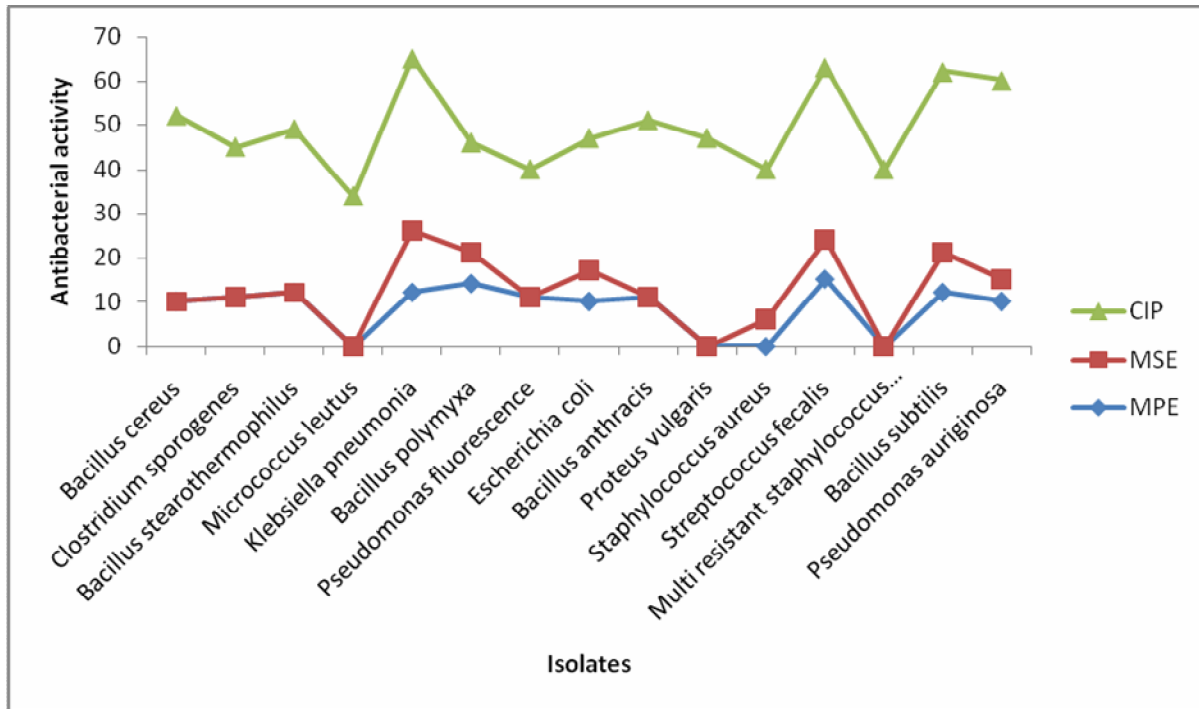


Figure 1: The antibacterial activity of methanol extract of *Prosopis africana*

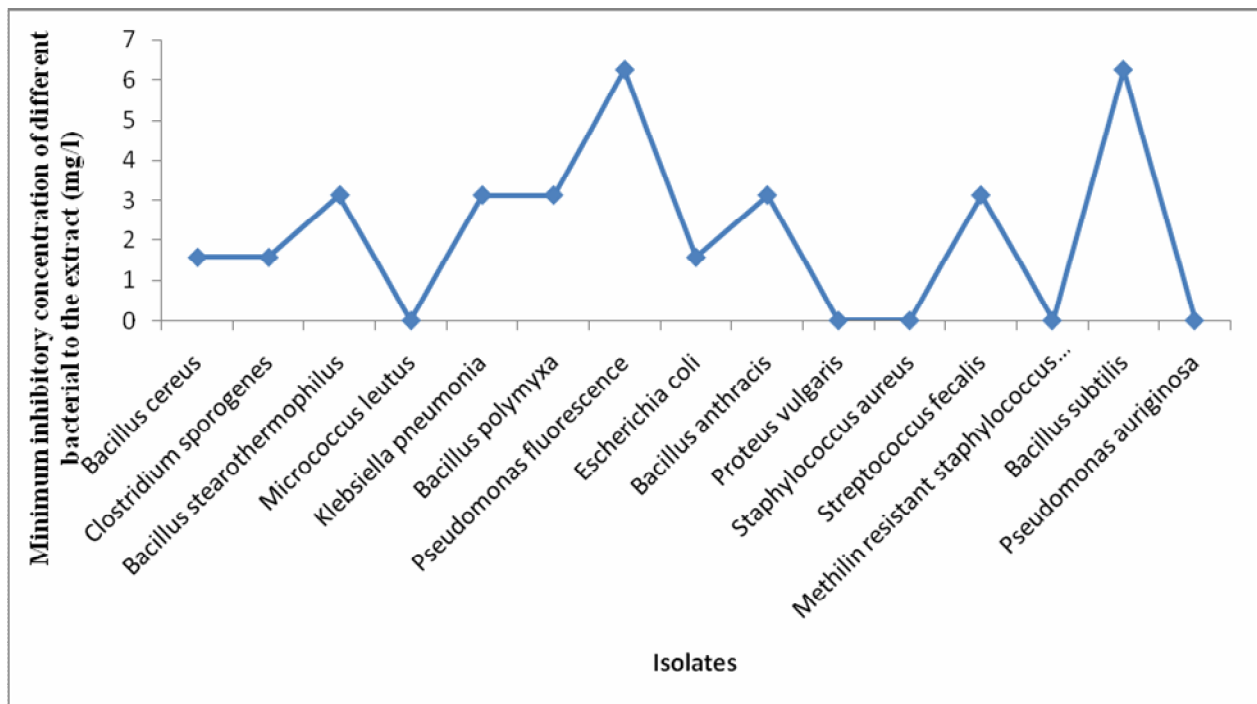


Figure 2: Minimum Inhibitory Concentration of bacteria

Table 1: Phytochemical composition of seed and pod extract of *Prosopis africana*

S/N	Tests	Seed extract	Pod extract
1	Phlobatannin	+	+
2	Flavonoid	+	-
3	Cyanoglycoside	-	+
4	Tannin	+	+
5	Saponin	+	+
6	Steroid	+	+
7	Alkaloid	+	+

KEYS: + = Present, - = Absent

Table 2: Proximate and Mineral and Proximate composition of *Prosopis africana* seed (mg/ml).

S/N	Mineral	Composition	Proximate composition
1	Na	110.7 ± 0.1	Carbohydrate(1.9±0.3)
2	K	617.5 ± 0.1	Crudeprotein(23.6±1.5)
3	Mg	420.1 ± 0.1	Moisture (3.3±0.0)
4	Ca	362.5 ± 0.2	Crude fibre (54.0±0.8)
5	P	196.4 ± 0.1	Ash content (4.4± 0.1)
6	Mn	36.2 ± 0.4	
7	Cu	46.2 ± 0.7	
8	Zn	22.4 ± 0.0	
9	Fe	15.5 ± 0.4	
10	Pb	0.0±0.0	

DISCUSSION

Infectious diseases account for high proportion of health problems, especially in the developing countries and 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. In the constant effort to improve the efficacy and ethics of modern medical practice, researchers are increasingly turning their attention to folk

medicine as a source of new drug (Olajuyigbe *et al.*, 2011).

Results obtained from antimicrobial activity showed that the extracts of *P. africana* have substantial inhibitory effects against most of the tested bacterial species. The pod extract was higher in suppressing the bacterial growth. The antimicrobial activity of the extract also might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane.

The Inhibition of each bacterial species to the extracts was indicated. Although most of the isolates were susceptible to the extracts, zones of inhibition that was $\geq 10\text{mm}$ were considered as good antimicrobial activity. All

the isolates were susceptible of ciprofloxacin used as control. The average inhibition zones of these organisms from the seed extract were less than those obtained from the pod extract of the plant.

It can be deduced from the results obtained that *Pseudomonas aeruginosa* was resistant to the seed extract of *P. africana* and the reason being that it has 5mm zone of inhibition since extracts from plants are considered active against bacteria only when the zone of inhibition is > 6 mm as stated by having all other bacterial isolates to be totally resistant.

The activities of all the extracts against both Gram-negative and Gram-positive bacteria agreed with the study that indicated that plant extracts are capable of inhibiting these two groups of bacteria (Jimoh *et al.*, 2008; Rahman *et al.*, 2009). These antibacterial activities against both Gram-positive and Gram-negative bacteria may be indicative to the presence of broad spectrum antibiotic compounds or general metabolic toxins in addition to the pharmacologically active metabolites (Kostova and Dinchev, 2005).

This observations may be due to the fact that phenolic compounds and their derivatives are considered as antiseptic agents causing cell protein denaturation and increasing the permeability of cell membranes. Feeny *et al.*, (1998) also, indicated that the mechanism of action of aromatic planar quaternary alkaloids in the extracts could be attributed to their ability to interchelate with DNA. Flavonoids activity may be explained to be a result of their complex ability with extracellular and soluble proteins as well as bacterial cell walls (Cowan and Steel, 1999) while more lipophilic flavonoids may disrupt microbial membranes. The antimicrobial actions of tannins have been associated with their ability to couple with polysaccharides (Ya *et al.*, 1988). They are also known to inactivate microbial adhesions, enzymes, cell envelope and precipitate microbial protein. The presence of some of these plant secondary metabolites in a significant amount in the investigated parts of *P. africana* may have conferred antimicrobial activity on both pod and seed extracts of the plant. In this regard, the presence of these phytochemicals in the extract may have been

responsible for inhibition exhibited by both pod and seed extracts. These compounds such as saponin, tannins,steroids, flavonoid and alkaloids are known to have antibacterial activity against pathogens and could be used traditionally for therapeutic purposes (Usman and Osuji, 2007). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, and flavonoids which have been found *in vitro* to have antimicrobial properties (Cowan 1999; Dahanukar *et al.*, 2000).

The mineral content results showed that potassium was found to be the most abundant. Magnesium was found to be the next highest mineral component of $420.1 \pm 0.1 \text{ mg}/100 \text{ g}$ sample. It had been reported that magnesium is an activation of many enzyme systems and maintains the electrical potential in nerves (Ferraro *et al.*, 1987). The seed was richer in potassium, magnesium, calcium and phosphorus, but values of copper, zinc, iron and manganese were low while lead was not detected. The mineral content in the seed compared favourably with that of soybean and cowpea, African yam bean and *Triticum durum*. This indicated that *P. africana* seed could be good as feed supplement. The ratio of sodium to potassium, Na/K, in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended (Ajiboye, 2009). The Na/K value which was calculated to be 0.18 was less than one which shows that *P. africana* would probably reduce high blood pressure. The Ca/P and Ca/Mg weight ratios were 3.76 and 0.86 respectively. The value of Ca/Mg is very close to the recommended value of 1.035. Food is considered 'good' if the Ca/P ratio is above one and 'poor' if the ratio is less than 0.543 Ca/P ratio. In the present study (3.76), was an indication that *P. africana* would serve as good source of minerals for bone formation. Some plants have been recorded to contain certain sugars which are biologically active against some diseases. Basically, some of these minerals constitute antimicrobial potency to various plants parts.

The result of proximate analysis of *P. africana* seed extract showed that low moisture content will afford a long shelf life for the seed. The quantity of crude protein (23.6+1.5%) was

high compared with crude proteins in other protein-rich foods such as soybeans, cowpeas and pigeon. The crude fibre of 3.3±0.0% was relatively low when compared to that of most legumes such as pigeon pea and cowpea. There is evidence that dietary fibre has a number of beneficial effects related to its indigestibility in the small intestine.

CONCLUSION

From the results obtained when compared to the antibiotic (ciprofloxacin) used; it could be concluded, that the extracts of both the pods and seeds obtained from *P. africana* were less effective than the standard antibiotics used. Although the methanol extract of the pods, on the other hand, was more effective than that of the seeds. The susceptibility of the various bacteria employed in this study indicated that *P. africana* is a plant that will be useful in the treatment of enteric diseases in which these bacterial species used in this study are associated. Therapeutically, infections caused by *B. polymyxa* would be more relieved with the use of *P. africana* extract. The use of the plant part in decoction would likely yield better antibacterial effects while *P. africana* could be a source of new antibiotic compounds. This study justifies the relevance of *P. africana* in the treatment of microbial infection and its uses in medicine.

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