

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF THE LEAVES OF AFRICAN LOCUST BEAN PLANT (*PARKIA FILICOIDEA* WELW.)**Iyamu, M. I., Ekozien, M.I. and Omoigberale, M.N.O.**

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ABSTRACT

Petroleum ether, Acetone, ethanol, n-hexane and aqueous (cold and hot) extracts of the leaves of African Locust Bean Plant (*Parkia filicoidea* Welw.) were tested against the following six bacterial isolates: *Staphylococcus aureus* NCTC 10788, *Bacillus subtilis*, *Streptococcus viridans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* using the agar well diffusion method. The crude powdered plant samples of leaves were subjected to phytochemical screening using standard methods to test for the presence of carbohydrates and reducing sugars, anthraquinones, cardiac and cyanogenetic glycosides, saponins, tannins flavonoids and alkaloids. The hexane and petroleum ether extracts of the leaves including the control solvents distilled water and dimethylsulphoxide (DMSO) exhibited no antibacterial activity. The aqueous (hot and cold) extracts of the leaves were generally active against the Gram positive bacteria *S. viridans* and *B. subtilis* including the control organism *S. aureus* NCTC 10788 with a zone of inhibition which ranged from 16.33 ± 1.20 mm to 22.90 ± 1.33 mm and 16.33 ± 0.67 mm to 21.03 ± 0.55 mm respectively. The minimum inhibitory concentration (MIC) for the bioactive extracts ranged from 2.50 to 15.00 mgml⁻¹. Acetone and ethanol leaves extract were active only against the Gram positive bacteria with a zone of inhibition which ranged from 15.00 ± 0.00 to 22.00 ± 1.15 mm. The test organisms were generally more sensitive to the commercial antibiotics gentamicin, ciprofloxacin and amoxicillin than the plant extracts with a zone of inhibition which ranged from 16.96 ± 0.09 mm to 29.30 ± 0.36 mm; *E. coli* and *K. pneumoniae* were not sensitive to amoxicillin. Results of the phytochemical screening showed that Carbohydrates, reducing sugars, saponins, tannins and flavonoids were present.

Keywords: Leave extract, minimum inhibitory concentration, antibiotics

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INTRODUCTION

Parkia filicoidea popularly called the African Locust Bean Tree, belongs to the family leguminosae. The leaves are alternate, dark green and bipinate with about 13 to 60 pairs of leaflets. The stem bark is dark greyish brown, thick and fissured. *Parkia* plants have been found to contain alkaloids, cellulose, lignins, inuline, saponins, tannins, starch and cyanogenic glycosides. The chemical constituents of many local drug plants including *Parkia* spp. are still being researched upon. Chemical analysis of such plants involves rather slow and time consuming research and very little progress has been made in this direction in Nigeria. Summarily, the three ways in which plants have been found useful in medicine are firstly, they may be used directly as tea or in other extract forms for their chemical constituents. Secondly, they may be used as agents in the synthesis of drugs. Finally, the organic molecules found in plants may be used as models for synthetic drugs. These plants are therefore finding use as pharmaceuticals, neutraceuticals, cosmetics and food supplements.

The use of plant extracts and biologically active compounds isolated from plant species have been widely reported in herbal medicine (Essawi and Srour, 2000). According to WHO (1995), there is a general agreement that poverty not only increases the risk of ill-health and vulnerability of people, it also has serious implications for the delivery of effective health-care such as reduced demand for services. This reduced demand is especially true for orthodox medicines. Hence millions of rural households use medicinal plants in a self help mode. The synthetic drugs are not only expensive and ineffective for the treatment of diseases but are with adulterations and side effects in the developing countries (Shariff, 2001).

Medicinal plants are used in preventive, promotive and curative applications, although in most cases no scientific studies have been done to prove the efficacy of these medicinal plants. Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethnobotanists, anthropologists, pharmaceutical chemists, physicians and microbiologists. Plant extracts contain some active components. The active ingredients of medicinal

plants are commonly more concentrated in organs like roots, seeds, barks and leaves but less in flowers while woods and woody parts of herbaceous plants are usually relatively inert (Iwu, 1993). These active ingredients are probably responsible for their antimicrobial activities. Ali *et al.* (1988) and Oyegade (1997) reported that some base compounds commonly associated with plants which have medicinal value include volatile oils, fats, resins, oleo-resins, steroids etc. Terpenes are amongst the chemicals responsible for the medicinal, culinary and fragrant uses of aromatic and medicinal plants (Dorman and Deans, 2000). In addition to these, plants are also known to synthesize a large variety of chemical substances referred to as secondary metabolites (Sofowora, 1993). These chemical substances include phenolic compounds, terpenes, steroids, alkaloids, glycosides, tannins, saponins, flavonoids and gums. Plants are known to produce some chemicals which are naturally toxic to bacteria and fungi (Basile *et al.*, 1999).

This study is aimed at increasing the knowledge of medicinal plants in Nigeria. This will go a long way to aid in the search for compounds in higher plants for the development of new drugs for use in medicine and agriculture. This research is therefore geared towards the provision of a scientific basis for the use of the plant *P. filicoidea* in traditional medicine in parts of Nigeria. The specific aims and objectives of this research includes: to determine the chemical constituents and basis for utilization of *P. filicoidea*; to compare the activities of the plant extracts against those of standard antibiotics; to identify the bioactive agents in the plant parts; and to determine possible antiseptic or disinfectant substances in the plant.

MATERIALS AND METHODS

Collection and processing of Plant Materials

Plant samples were collected during the dry season from October 2005 to January, 2009 from Ekpoma, Esan West Local Government Area and Ewu, Esan Central Local Government Area of Edo State, Nigeria. The leaves were removed from the branch and dried whole without cutting. The plant parts were further dried in an oven at 50°C for two to seven day before pounding separately in a clean mortar with pestle into coarse powder. The coarse powder was further milled in a milling machine (Viking, Exclusive Joncod, USA.) and then stored separately in large glass bottles with air tight covers and were kept at room temperature of $28 \pm 2^{\circ}\text{C}$ until required

Preparation of Plant Extracts

Six different solvents namely; hot water (100°C), cold water (ambient temperature of 28 ± 2°C), n-hexane, acetone, ethanol and petroleum ether were used to obtain extracts from the plant parts. Distilled water and dimethyl sulphoxide (DMSO) were used as control. Plant extracts were obtained by maceration as described by Abdelouaheb *et al.* (2006)

Test Organisms

The test organisms used were clinical isolates obtained from the Pathology Laboratory of the University of Benin Teaching Hospital, Benin-City. The Gram positive organisms were *Bacillus subtilis* and *Streptococcus viridans* while the Gram negative organisms were *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The organisms were reconfirmed by purification, characterization and identification according to the standard methods of Cowan and Steel (1993), Buchanan and Gibbons (1984). *Staphylococcus aureus* (NCTC 10788) was used as control.

Screening of Extracts of *Parkia filicoidea* for Antibacterial Activity

The agar well diffusion method as described by Irobi *et al.* (1996) and Okeke *et al.* (2001) was used for the sensitivity test. A pure culture of each test organism was grown in nutrient broth (Oxoid) for 18 hours at 37°C. The broth culture was standardized to match McFarland turbidity standard which was approximately 1.0×10^8 cfu/ml as described above. This inoculum was used to seed 20ml. of cooled molten nutrient agar medium in Petri dishes using an automatic micropipette. A sterile cork borer (8mm in diameter) was then used to dig wells equidistant from each other on the surface of the solidified nutrient agar medium. The bases of the wells were sealed with 0.02ml of molten nutrient agar and 0.25ml of each extract were delivered into wells. As control, 0.25ml of sterile distilled water and 0.25 ml of dimethyl sulphoxide (DMSO) were used. *S. aureus* (NCTC 10788) was used as control for the test organisms. The plates were then incubated at 37°C for 24 hours after which zones of inhibition present were measured

For comparison, commercial antibiotics discs (Gentamicin (10 µg/ml, Oxoid), Ciprofloxacin (5 µg/ml, Oxoid) and Amoxicillin (25 µg/ml, Oxoid) were applied to separate plates seeded with the test organisms. Zone of inhibition diameter > 6mm indicated activity while < 6mm showed lack of activity by the plant extract (Mudi and Ibrahim, 2008).

Determination of Minimum Inhibitory Concentration (MIC)

The plant extracts used for MIC were hot water, cold water, acetone and ethanol extracts of the leaves of the plant. The concentrations were 20mg/ml, 15mg/ml, 10mg/ml, 7.5mg/ml, 5.0mg/ml, 2.5mg/ml. Plates without plant extracts were used as positive control. A loopful of the organisms previously diluted to 10^8 cfu/ml was used to inoculate the plates which were incubated at 37°C for 24 hr. The MIC of the extract was regarded as the lowest concentration that did not permit growth of the test organism (Alade *et al.*, 1986).

Statistical Analysis

The data obtained for the research were subjected to single analysis of variance test.

Phytochemical Screening

Phytochemical tests for carbohydrates and reducing sugars, saponins, tannins, flavonoids, anthraquinones, cardiac and cyanogenetic glycosides were carried out according to standard methods (Harborne, 1993; Trease and Evans, 2002).

RESULTS

The antibacterial activities of the acetone, ethanol, n-hexane, petroleum ether and aqueous (cold and hot) extracts of the leaves of the African Locust Bean plant (*P. filicoidea* Welw.) on *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus viridans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* is shown in Tables 1.

Table 1: Antibacterial activity of leaves extract of *Parkia filicoidea* Welw. and commercial antibiotics on the test organisms

Organisms	Mean diameter of zone of inhibition (mm ± SE)										
	Hot Water	Cold Water	Acetone	Ethanol	Hexane	Petroleum ether	CIP 5µg	GN 10µg	AMX 25µg	DMSO 0.25ml	Dist. H ₂ O 0.25ml
+ <i>Bacillus subtilis</i>	21.50±0.87	18.00±0.00	19.00±1.15	17.00±1.73	0.00	0.00	20.29±0.39	24.37±0.77	16.96±0.09	0.00	0.00
+ <i>Streptococcus viridians</i>	22.33±1.01	21.03±0.55	19.00±1.15	15.00±0.00	0.00	0.00	19.48±0.46	21.45±0.35	17.97±0.29	0.00	0.00
* <i>Escherichia coli</i>	16.33±1.20	16.33±0.67	0.00	0.00	0.00	0.00	29.30±0.36	20.65±0.50	0.00	0.00	0.00
* <i>Pseudomonas aeruginosa</i>	0.00	0.00	0.00	0.00	0.00	0.00	27.18±0.20	21.32±0.57	19.85±0.82	0.00	0.00
* <i>Klebsiella pneumoniae</i>	0.00	0.00	0.00	0.00	0.00	0.00	26.90±0.15	24.93±0.28	0.00	0.00	0.00
+ <i>Staphylococcus aureus</i> NCTC 10788 (Control)	22.90±1.33	18.00±0.58	22.00±1.15	21.87±0.43	0.00	0.00	24.37±0.81	28.82±0.64	19.60±0.35	0.00	0.00

Each value is the mean activity of the three strains for each test organism; test concentration of extract = 100mg/ml

Key

+ Gram positive bacteria; * Gram negative bacteria; 0 No zone of inhibition; CIP = Ciprofloxacin; GN = Gentamicin; AMX = Amoxicillin; DMSO = Dimethylsulphoxide

Table 2: Minimum Inhibitory Concentration (MIC) of the leaves extract of *P. filicoidea* Welw.

Organisms	Minimum inhibitory concentration (mg/ml)					
	Hot Water	Cold Water	Acetone	Ethanol	Hexane	Petroleum ether
+ <i>Bacillus subtilis</i>	2.50	7.50	7.50	5.00	ND	ND
+ <i>Streptococcus viridians</i>	2.50	2.50	7.50	15.0	ND	ND
* <i>Escherichia coli</i>	10.0	10.0	ND	ND	ND	ND
* <i>Pseudomonas aeruginosa</i>	ND	ND	ND	ND	ND	ND
* <i>Klebsiella pneumonia</i>	ND	ND	ND	ND	ND	ND
+ <i>Staphylococcus aureus</i> NCTC 10788 (Control)	2.50	7.50	2.50	2.50	ND	ND

Each value is the mean activity of the three strains for each test organism; test concentration of extract = 100mg/ml

Key

+ Gram positive bacteria; * Gram negative bacteria; ND Not determine

Table 3: Percentage Yield of crude leaves extracts of *P. filicoidea* from hot water, cold water, acetone, ethanol, hexane and petroleum ether

Extraction solvent	Colour of filtrate	Yield (g)	Yield ± SE (%)
Hot water	Dark Brown	1.80	4.49 ± 0.01
Cold water	Light Brown	2.10	5.40 ± 0.06
Acetone	Green	4.60	1.70 ± 0.06
Ethanol	Dark Green	3.03	7.70 ± 0.04
Hexane	Yellow	1.10	2.33 ± 0.06
Petroleum ether	Light Yellow	0.50	1.40 ± 0.06

Each value is a mean of triplicates

The results of the phytochemical screening of *P. filicoidea* are shown in Table 4. The plant part analyzed, consisted mainly of carbohydrates and reducing sugars, saponins, tannins and flavonoids. Anthracene derivatives, alkaloids, cardiac and cyanogenetic glycosides were absent.

Table 4: Phytochemical components of crude leaves extracts of *P. filicoidea*

Phytochemical component	Hot water	Cold water	Acetone	Ethanol	Hexane	Petroleum ether
	Carbohydrates	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+
Anthracene derivatives	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-
Cyanogenetic glycosides	-	-	-	-	-	-
Tannins	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+

Key

+ = Present - = Absent

DISCUSSION

All the test organisms were not sensitive to n-hexane, petroleum ether extract, distilled water and dimethyl sulphoxide (DMSO) used as control (Tables 1). This may probably be because these organic solvents were not appropriate for the extractions of the active principles present in the plant. This is not surprising since hexane and petroleum ether generally had the lowest yields from the different parts investigated (Table 3).

The result is in contrast to that of Obafemi *et al.* (2006) who found that the hexane extract of *Tithonia diversifolia* (Mexican sunflower) leaves inhibited *B. subtilis*, *E. coli* and *K. pneumoniae*. This result is similar to theirs in that they also reported that *S. aureus* and *P. aeruginosa* were not sensitive to the hexane extract of the leaves of *Tithonia diversifolia*. All the organisms just mentioned were also found not to be sensitive to the extracting solvent DMSO (Obafemi *et al.*, 2006). This result is also in contrast to that of Erturk *et al.* (2003) who reported that the n-hexane extract of *Viscum album L. sub sp. abietus* (Wiesb.) showed antimicrobial activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *Enterobacter cloacae*, *Proteus vulgaris* and *Candida albicans*.

The reason the hot water extract of the leaves was generally more active than the cold water extract may be that heat is important in the extraction due to the nature of the leaves which are soft (Table 1). Though the acetone extract of the leaves was generally more active than the ethanol extract of the leaves (Table 1), the percentage yield of the crude extract of the leaves for ethanol (7.70 ± 0.04) was far higher than that for acetone (1.70 ± 0.06) (Table 3). Hence yield of the extract may not necessarily correlate with the activity of the plant.

However, there were no significant differences in the mean zones of inhibition for the aqueous extracts of the leaves against *S. viridans* and *E. coli* ($P > 0.05$). There was also no significant difference in the percentage yield obtained for the hot water and cold water extracts of the leaves ($P > 0.05$) (Table 3). The aqueous extracts (hot and cold) and acetone extracts of the leaves were more active on the Gram positive bacteria than the ethanol extracts (Table 1). The active extracts of the leaves generally had a narrow spectrum of activity.

The difference in the mean diameter of zone of inhibition produced by the ethanol leaf extract was not significant ($P > 0.05$) for *S. viridans*. The differences in the mean diameter zones of inhibition produced by the hot water compared to all the other active extracts against *S. aureus* were significant ($P < 0.05$). Generally, these plant extracts inhibited the Gram positive organisms better than the Gram negative ones. This is in agreement with previous reports that plant extracts are more

active against Gram positive bacteria than Gram negative bacteria (Lin *et al.*, 1999; Vlietinck *et al.*, 1995; Rabe and Van Staden, 1997).

The range of MIC values for the test organisms correlated with the results obtained for the antibacterial activity. The MIC values for the hot water extract of the leaves against the Gram positive bacteria tested were lower than for *E. coli*. The MIC values for cold water against *S. viridans*, acetone and ethanol extracts against *S. aureus* were also lower (2.50 mg/ml) when compared to the aqueous extracts (hot and cold) of the leaves (10.00 mg/ml) against *E. coli*. This shows that the Gram positive bacteria were more susceptible to the effect of the hot water extracts of the leaves. *S. aureus* and *B. subtilis* were also more susceptible to the ethanol extract of the leaves than the cold water leaves extract.

The test organisms were generally more sensitive to the standard antibiotics gentamicin, ciprofloxacin and amoxicillin when compared to the active plant extracts. Gentamicin was the most active when compared to ciprofloxacin and amoxicillin especially against the Gram positive bacteria *S. aureus* NCTC 10788, *B. subtilis* and *S. viridans*.

However, for the Gram negative bacteria, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, the reverse was the case where ciprofloxacin showed better activity when compared with gentamicin (Tables 1). Gentamicin also showed the best activity when compared to the plant extracts against both the Gram positive and Gram negative bacteria except for the aqueous extracts (cold and hot water) against *S. viridans*.

This is similar to the data presented by other scholars (De and Ifeoma, 2002; Kubmarawa *et al.*, 2002). This may be due to the fact that while conventional antibiotics and non-antibiotic antibacterial agents were usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from plant and animal origins and most of the time are subjected to contamination and deterioration (De and Ifeoma, 2002). Amoxicillin was not as active as most of the plant extracts and did not show any activity against the Gram negative bacteria *E. coli* and *K. pneumoniae* for all the plant parts investigated.

This study however showed that the aqueous extracts (hot and cold) gave relatively higher yield when compared to the hexane and petroleum ether extracts (Table 3). According to Doughari *et al.* (2008); Kamba and Hassan (2010) water was better than ethanol as solvent used in the studies of the antibacterial activity of leaf extracts of *Senna obtusifolia* and *E. balasamifera* respectively. El-Mahmood (2009) also found water to be the best of all the solvents used for his analysis in the study of antibacterial activity of crude extracts of *E. hirta*.

The results of this research also showed that the leaves of *P. filicoidea* investigated possessed important secondary metabolites like carbohydrates and reducing sugars, saponins, tannins

and flavonoids in varying quantities except anthraquinones cardiac and cyanogenetic glycosides and alkaloids (Table 4). Apart from carbohydrates, reducing sugars and flavonoids which are present in appreciable amounts in the plant parts investigated, the leaves and stem bark had moderate amounts of saponins and tannins (Table 4).

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