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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF THE STEM BACK OF AFRICAN LOCUST BEAN PLANT (*PARKIA FILICOIDEA* WELW.)

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Abstract

Acetone, ethanol, n-hexane, petroleum ether and aqueous (cold and hot) extracts of the stem bark of African Locust Bean Plant (*Parkia filicoidea* Welw.) were tested against 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 the stem bark 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 stem bark including the control solvents distilled water and dimethylsulphoxide (DMSO) exhibited no antibacterial activity. The aqueous (hot and cold) extracts of the stem bark 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 17.00 ± 1.73 mm to 19.30 ± 0.46 mm and 17.67 ± 0.67 mm to 23.17 ± 1.51 mm respectively. Acetone and ethanol extracts were active against both the Gram positive and Gram negative organisms *E. coli* and *P. aeruginosa* and *K. pneumoniae* with a zone of inhibition which ranged from 18.33 ± 2.32.58 mm to 21.50 ± 0.00 mm and 13.00 ± 0.00 mm to 20.00 ± 2.08 mm respectively. The minimum inhibitory concentration (MIC) for the bioactive extracts ranged from 2.50 to 20.00 $mgml^{-1}$. 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. The results of the phytochemical screening showed that carbohydrates, reducing sugars, saponins, tannins and flavonoids were present.

Keywords: Stembark, Phytochemical, Acetone, MIC.

Introduction

The use of plants as source of medicine has been reported in virtually all cultures of the world. Plants extracts and biologically active compounds isolated from plant species have also 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 their efficacy. Modern approaches to determine the medicinal properties of plants and antibacterial activities of plant extracts 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 and steroids. 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 also known to produce some chemicals which are naturally toxic to bacteria and fungi (Basile *et al.*, 1999).

Summarily, the three ways in which plants have been prepared as 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.

This study is therefore 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 the 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 stem bark 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 fresh stem bark were thoroughly washed with clean water and cut into tiny bits before they 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 in large glass bottles with air tight covers and were kept at room temperature of 28 ± 2^0C until required.

Preparation of Plant Extracts

Six different solvents namely;hot water (100^0C), cold water (ambient temperature of 28 ± 2^0C), 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 (l993), Buchannan 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 x $l0^8$ cfu/ml. This inoculum was used to seed 20ml. of cooled molten nutrient agar medium in Petri dishes. 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 0.02m1 of molten nutrient agar and 0.25ml of each extract was 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 37^0C 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 stem bark. The concentrations were 20mg/ml, 15mg/ml, 10mg/ml, 7.5mg/ml, 5.0mg/ml and 2.5mg/ml. Plates without plant extracts were used as positive control.

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 stem bark of the African Locust Bean plant (*P. filicoidea* Welw.) on *Staphylococcus aureus, Bacillus subtilis, Streptococcus viridans, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are shown in Table 1.

Table 1: Antibacterial activity of stembark extract of *P. 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 5 g**	**GN 10 g**	**AMX 25 g**	**DMSO 0.25ml**	**Disti. H_2O 0.25ml**
+ *Bacillus subtilis*	19.30±0.46	17.67±0.67	18.33±2.32	17.00±0.00	0.00	0.00	20.39±0.39	24.37±0.77	16.96±0.09	0.00	0.00
+ *Streptococcus viridans*	17.17±0.17	18.00±0.00	20.17±0.69	16.00±0.00	0.00	0.00	19.48±0.46	21.45±0.35	17.97±0.29	0.00	0.00
* *Escherichia coli*	0.00	0.00	0.00	16.00±0.00	0.00	0.00	29.30±0.36	20.65±0.50	0.00	0.00	0.00
* *Pseudomonas aeruginosa*	16.00±0.58	13.00±0.00	20.00±0.00	13.00±0.00	0.00	0.00	27.18±0.20	21.32±0.57	19.85±0.82	0.00	0.00
* *Klebsiella pneumoniae*	16.00±0.58	0.00	21.00±0.00	15.13±0.13	0.00	0.00	26.90±0.15	24.93±0.28	0.00	0.00	0.00
+ *Staphylococcus aureus* **NCTC 10788 (Control)**	17.00±1.73	23.17±1.51	21.50±0.00	20.00±2.08	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 stembark extracts of *P. filicoidea* Welw.

Organisms	*Minimum inhibitory concentration (mg/ml)*					
	Hot Water	**Cold Water**	**Acetone**	**Ethanol**	**Hexane**	**Petroleum ether**
+ *Bacillus subtilis*	2.50	7.50	7.50	10.00	ND	ND
+ *Streptococcus viridians*	2.50	7.50	2.50	10.00	ND	ND
* *Escherichia coli*	ND	ND	ND	10.00	ND	ND
* *Pseudomonas aeruginosa*	15.00	15.00	2.50	20.00	ND	ND
* *Klebsiella pneumonia*	15.00	ND	2.50	5.00	ND	ND
+ *Staphylococcus aureus* NCTC 10788 (Control)	2.50	2.50	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 stembark extracts of *P. filicoidea*

	Extraction solvent	Colour of filtrate	Yield (g)	Yield ± SE (%)
	Hot water	Brown	1.49	3.80 ± 0.01
	Cold water	Brown	1.20	3.00 ± 0.01
	Acetone	Dark Brown	1.90	4.86 ± 0.01
	Ethanol	Dark Brown	2.77	7.00 ± 0.35
	Hexane	Light Yellow	0.19	0.49 ± 0.01
	Petroleum ether	Light Yellow	0.10	0.30 ± 0.01

Each value is a mean of triplicates

The results of the phytochemical screening of *P. filicoidea* stembark extracts are shown in Table 4. The stembark 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

Table 5: Proximate analysis of the phytochemical constituents of stembark extract of *P. filicoidea*

Components	**Plant Parts / Yield ± SE (mg/100g)**
	Stem bark
Carbohydrate	58.60±0.07
Sugar	6.00±0.07
Saponin	19.08±0.09
Tannin	83.00±0.63
Flavonoid	125.74±3.40
Anthracene derivatives	ND
Cardiac glycoside	ND
Cyanogenetic glycosides	ND
Alkaloid	ND

Each value is a mean of triplicates.

Key

ND - Not Determined

Discussion

All the test organisms were not sensitive to n-hexane, petroleum ether extract, distilled water and dimethyl sulphoxide (DMSO) used as control (Table 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 stembark extracts (cold and hot water, acetone and ethanol) were active against both the Gram positive and Gram negative organisms except *E. coli* (Table 1). Hence, the stembark extracts of the plant *P.filicoidea* showed a broad spectrum of activity against the test organisms (Table 1). Acetone stembark extracts generally, just like those of the leaves again were more active on the test organisms than ethanol stembark extracts with a wide range of zones of inhibition 16.00 ± 0.00 mm to 20.17 ± 0.69 mm against *S. viridans*, 13.00 ± 0.00 mm to 20.00 ± 0.00 mm against *P. aeruginosa* and 15.13 ± 0.13 mm to 21.00 ± 0.00 mm for *K. pneumoniae* (Table 1).

With the aqueous extracts (hot and cold) of the stem bark, the nature of the antibacterial activity against the test organisms varied. It could not be stated that hot water was more active than the cold water on the test organisms or vice versa. These variations could be as a result of various factors like the mode of extraction, the amount of solvent used for the extraction and even the nature of the stem bark which is quite different from that of the leaves. The MIC values of the hot water stem bark extracts against the Gram positive bacteria were also lower (2.50 mg/ml) than for the Gram negative bacteria (15.00 mg/ml) *P. aeruginosa* and *K. pneumoniae* hence the Gram positive bacteria were also more susceptible to the effects of this extract than the Gram negative.

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 4). 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 stem bark of *P. filicoidea* investigated possessed important secondary metabolites like carbohydrates, reducing sugars, saponins, tannins and flavonoids in varying quantities except anthraquinones, cyanogenetic glycosides and alkaloids (Table 4 and 5).

Conclusion

In this era of increasing microbial resistance to standard antibiotics and with the high costs of synthetic drugs, the crude extracts of this plant *P. filicoidea* Welw. can be used as an alternative in medicine. Thus there is need to investigate the cost effectiveness of using the plant extracts for management of diseases where they are effective instead of using standard antibiotics which are more expensive. The need to investigate the effect of the presence of the phytochemicals on the physiological status of the consumers is necessary and isolation of the active ingredients and characterization to elucidate their structures and functional group(s) present are recommended for further research.

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