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Antimicrobial Activity and Phytochemical Screening of Five Selected Seeds from Nigeria

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ABSTRACT

The phytochemical screening and antimicrobial activity of five selected seeds (*Enterolobium cyclocarpum*, *Delonix regia*, *Mucuna flagellipes*, *Adenanthera pavonina* and *Pentaclethra macrophylla*) from Nigeria was carried out. Some secondary metabolites such as alkaloid tannin, saponin, flavonins, anthraquinones, phenols, phlobatannins, chalcones, steroids, terpenes cardenolides and glycosides were detected in these seeds at different concentrations ranging from 0.229 to 3.524%. These seed extracts showed inhibitory activity against all tested microorganisms except for *E. cyclocarpum* and *M. flagellipes*. This study suggests that the aqueous extracts from these seeds could be explored as possible antimicrobial agents.

Keywords: bioactive agents, extracts, inhibition, metabolites, microorganisms

INTRODUCTION

Natural products, has the name implies, are those chemical compounds derived from living organisms, plants, animals, insects and the study of natural product is the investigation of their nature, formation, use and purpose in the organisms. Ancient man is known to have utilized plants as drugs for millennia when confronted with illness and diseases (Sofowora 1993). Herbal medicine in the simplest form is medicine or drug made from herbs of plants and can so be said to possess several synonyms all of which refers to plants as the raw materials for medicine. Increasingly, the world is returning to nature in the treatment and management of common prevalent diseases affecting man (Fransworth *et al*. 1993). It is therefore important that herbal medicine should be of uniform quality and identity, the chemical composition as well as the efficacy to initiate a comprehensive study of herbal medicine needs to be investigated.

This present research work aimed at investigating the phytochemical, proximate and *in vitro* antimicrobial properties of five medicinal plant seeds from Nigeria. These seeds belong to the Leguminosae family and they are: *Enterolobium cyclocarpum*, *Delonix regia*, *Mucuna flagellipes*, *Adenanthera pavonina* and *Pentaclethra macrophylla.*

MATERIALS AND METHODS

Plant material and extraction

The plant samples were collected at the University of Ibadan and authenticated at the Botany and Microbiology Department of the Faculty of Science University of Ibadan. The seeds from the plants were air dried, powdered and extracted with ethanol in a Soxhlet extractor for 18-20 h. The extracts were concentrated after the extraction and preserved in the refrigerator at 4°C.

Phytochemical tests

The different extracts were tested for the presence of chemical constituents using standard methods.

1. Alkaloids

0.5 g each of the dried ethanol extracts were weighed and re-extracted with 5 ml of 5% hydrochloric acid. The hydrochloric acid extract were filtered. Few drops of Drangendruff reagent were added to 2.5 ml of the filtrate. A reddish-brown color and turbidity with the reagent indicates the presence of alkaloid. The concentration was determined using the method described by Henry (1993).

2. Tannins

0.5 g of the dried and powdered seed samples were stirred with 10 ml of distilled water, filtered and ferric chloride was added to the filterate. Appearance of blue or blue-black, green or blue-green coloration showed the presence of tannin. Quantitative determination was also carried out (Swain 1979)

3. Saponins

0.5 g of each of the samples was shaken with 10 ml of distilled water in test tube. Persistent frothing was taken as preliminary evidence of saponin (Henry 1993).

4. Flavonoids

0.5 g of each of the ethanol extracts were separately treated with four drops of concentrated hydrochloric acid after which 0.5 g of Magnesium turning was added. Development of pink of magenta-red coloration indicates the presence of flavonoid (Murugan and Kathaperumal 1987).

5. Anthraquinones

0.5 g of the powdered seed samples were shaken with 10 ml of benzene and then filtered, followed by the addition of ammonium hydroxide to the filterate. The formation of a pink, red or violet coloration in the ammoniacal phase indicates the presence of anthraquinone (Harborne 1973).

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Short Communication

Table 1 Qualitative phytochemical screening of the aqueous extracts of five medicinal seeds.

Organisms	AL	TA	SA	FL	AN	PHE	PH	CH	ST	TE	CAR	GL
E. cyclocarpum	++	+	+	++	-	+++	+	++	++	+	-	-
D. regia	++	+	-	-	++	++	+	+	+	++	++	-
M. flagellipes	+++	+	+	-	++	++	+	-	+	+	+	-
A. pavonina	+++	+++	-	++	-	++	++	-	+++	++	-	-
P. macrophylla	++	++	++	+	-	+++	++	-	+++	++	++	-

+++ = Present in an appreciable amount
++ = Present in a moderate amount
+ = Present in a trace amount or minute amount
- = Completely absent
AL = Alkaloid, AN = Anthraquinones , CAR = Cardenolides , CH = Chalcones , , FL = Flavonins , GL = Glycosides., PH = Phlobatannins, PHE = Phenols, SA = Saponin , ST = Steroids, TA = Tannin, TE = Terpenes

Table 2 Quantitative phytochemical screening of the aqueous extracts of five medicinal seeds.

Sample	Alkaloid (%)	Tannin (%)	Phenol (%)	Phlobatannin (%)	Steroids (%)
E. cyclocarpum	1.187	2.597	3.498	0.229	1.522
D. regia	2.008	2.647	2.805	0.240	1.607
M. flagellipes	2.866	2.609	2.875	0.236	1.414
A. pavonina	2.045	2.665	2.915	0.286	1.609
P. macrophylla	1.132	2.833	3.524	0.241	1.674

AN = *Aspergillus niger*, BS = *Bacillus subtilis*, CA = *Candida albican*, Ecoli = *Escherichia coli*, PA = *Pseudomona aeroginosa*, SA = *Staphylococcus aureus*

6. Phenols

0.5 g of each of the ethanol extracts were first extracted with ethyl acetate and the extracts were filtered. The development of a blue-black or brown coloration on the addition of ferric chloride indicates the presence of phenol (Trease and Evans 1983). This was also determined quantitatively using the method described by Swain (1979).

7. Phlobatannins

0.5 g of the aqueous extract of the seed sample were boiled with 1% hydrochloric acid. The presence of phlobatannin was indicated by the deposition of a red precipitate (Harborne 1973). Spectrophotometry was used to determine the concentration (Henry 1993).

8. Chalcones

2 ml of ammonia solution was added to 5 ml of the ethanol extract of each of the seed samples. Formation of a reddish color confirms the presence of chalcone (Polk 1996).

9. Steroids

2 ml of acetic anhydride was added to 0.50 g of each of the seed samples and cooled in the ice. 1 ml of concentrated sulphuric acid was carefully added three times to obtain a color change from violet to blue and blue to finally green. This color change confirms the presence of steroid. The spectrophotometry determination was achieved using the method of Wall *et al*. (1952).

10. Terpenes

Liebermans Burchard's reagent (mixture of 10 ml of acetic anhydride, concentrated sulphuric acid and 20 ml chloroform) was added to 0.50 g of each of the seed sample. A bluish-green precipitate indicates the presence of terpenes (Trease and Evans 1983).

11. Cardenolide

0.50 g of each powdered seed samples were added to 2 ml of glacial acetic acid containing one drop of ferric chloride solution. 1 ml of concentrated sulphuric acid was added to underlay it. A brown ring precipitate obtained indicates the presence of cardenolide.

12. Glycosides

0.5 g of each powdered samples were dissolved in 2 ml of chloroform. 10 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interphase indicates the presence of glycoside (Rahila *et al*. 1994).

Organisms and media

Micoorganisms used in the present study were mold (*Aspergillus niger* and *Candida albicans*), gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Gentamicin and Tioconazole were used as bacteria and fungi positive control, respectively. The microorganisms were cultured in Sabouraud dextrose agar.

Antibacterial activity

Aqueous extracts of the seeds were used for the antimicrobial test. Agar-well diffusion method was used. The wells were marked and the prepared serial dilution of each of the sample was put into the wells and allowed to diffuse properly for about 45 min. All bacteria plates were incubated for 24 h at 37°C while the fungi plates were incubated for 48 h at 28°C. The clear zone of inhibition was observed and the readings were taken accordingly. Different concentration strengths of the extracts were prepared which ranged from 3.125 to 100% in order to determine the inhibition concentration strength of each of the extracts.

RESULTS AND DISCUSSION

Table 1 presents the qualitative phytochemical screening of five medicinal plants from Nigeria. Phenol, alkaloid, steroid, terpene and tannin are dominant in the studied plants. Chalcone and anthraquinone are present in small amount while glycoside was not found in any of the aqueous extracts. Anthraquinone is only present in *D. regia* and *M. flagellipes* in moderate amount while chalcone was only in present in *E. cyclocarpum* and *D. regia* in moderate and minute amount respectively. Alkaloid, tannin, phenol, phlobatannin, steroid and terpene were all detected in all the aqueous extracts analyzed. Keto and amino acids have been isolated from the flowers of *D. regia* (Mukherjee 1975). Polyphenols were also reported from the flowers of same *D. regia* (Felix *et al*. 2008) The results of this present study also shows the presence of the families of these reported isolated compounds. The antiprotozoal effect of the foliage of *E. cyclocarpum* has been examined by Ivan *et al*. (2003). They found it to be positive in this respect. This effectiveness may be accounted for as a result of the presence of these secondary metabolites present in it. Alkaloid, tannin, flavonoid and saponin are known to show medicinal activity as well as physiological activity (Sofowora 1993). The presence of steroidal compounds in these seeds suggests their usefulness in pharmacy since these steroidal compounds serve as potent starting materials in the synthesis of sex hormones (Okwu 2001). The presence of these determined groups of compounds indicates the possibility of these ex-

Table 3 Antimicrobial screening of the aqueous extracts of five medicinal seeds.

Sample	SA	BS	EC	PA	CA	AN	Control
E. cyclocarpum	+	+	+	-	+	+	+
D. regia	+	+	+	+	+	+	+
M. flagellipes	+	+	+	-	+	+	+
A. pavonina	+	+	+	+	+	+	+
P. macrophylla	+	+	+	+	+	+	+

AN = *Aspergillus niger*, BS = *Bacillus subtilis*, CA = *Candida albicans*, EC = *Escherichia coli*, PA = *Pseudomona aeroginosa*, SA = *Staphylococcus aureus*

Table 4 Concentration strength of the aqueous extracts of five medicinal seeds.

Sample	SA	BS	EC	PA	CA	AN
E. cyclocarpum	12.50	12.50	12.50	-	25.00	25.00
D. regia	12.50	12.50	12.50	100.00	25.00	25.00
M. flagellipes	12.50	12.50	12.50	-	25.00	25.00
A. pavonina	12.50	12.50	12.50	100.00	25.00	25.00
P. macrophylla	12.50	12.50	12.50	100.00	25.00	25.00

AN = *Aspergillus niger*, BS = *Bacillus subtilis*, CA = *Candida albicans*, EC = *Escherichia coli*, PA = *Pseudomona aeroginosa*, SA = *Staphylococcus aureus*

tracts being used as antimicrobial agents. The result of the quantitative analysis of these detected metabolites is shown in **Table 2**. This is the quantification of the amount of these detected secondary metabolites in the seeds. Phenol had the highest concentration among all the metabolites determined. It was found to be 3.524% in *P. macrophylla*. The concentration of phlobatannin ranged from 0.225-0.287%. It was found to be highest in *A. pavonina* with a concentration of 0.286% and least in *E. cyclocarpum* being 0.229%. The concentration of phenol was the highest in the studied samples while phlobatannin was the least.

The antimicrobial screening of the extracts showed that all the extracts are active against the growth of all the test organisms except for *E. cyclocarpum* and *M. flagellipes* against *P. aeruginosa* as shown in **Tables 3** and **4**. This susceptibility test suggests that these extracts could serve as antimicrobial agents. The concentration strength of the extracts was found constant at different concentration for the organisms tested and also throughout except for *P. aeruginosa*.

The antimicrobial activities of these extracts could be attributed to the presence of the metabolites in them as shown in **Table 1**, especially the presence of metabolites like tannin, alkaloid, phenol, glycoside, anthraquinone and flavonin (Chung *et al*. 1998).

PERSPECTIVE

A study was carried out on the phytochemical screening and antimicrobial activity of some medicinal seeds from Nigeria. The studied seed extracts showed some antimicrobial activities against some pathogenic organisms. This study suggests that these seeds have the potential of being sources of antimicrobial agents. However, a further study needs to be carried out on the isolation and identification of the precise bioactive compounds responsible for the activities exhibited by the extracts from these studied seeds.

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