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Phytochemical Screening and Antimicrobial Activity of 10 Medicinal Seeds from Nigeria

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ABSTRACT

The phytochemical screening and antimicrobial activity of 10 medicinal seeds (*Albizzia lebbeck*, *Strychnos spinosa*, *Myristica fragrans*, *Monodora myristica*, *Aframomum melegueta*, *Croton penduliflorus*, *Blighia sapida*, *Antiaris africana*, *Thevetia nerifolia* and *Terminalia catappa*) from Nigeria was carried out. The study revealed the presence of some secondary metabolites such as alkaloids, tannin, saponin, flavonins, anthraquinones, phenols, phlobatannins, chalcones, steroids, terpenes, cardenolides and glycosides. These metabolites are present in the seeds at different concentrations ranging from 0.21 to 3.67%. The study also showed inhibitory activity against tested microorganisms, all of them being active against *Candida albicans* and *Aspergillus niger* at a concentration of 25%. Some of the extracts tested did not show activity against *Pseudomonas aeruginosa*. This study suggests that the aqueous extracts from these seeds could be explored as possible antimicrobial agents.

Keywords: antimicrobial activity, bioactive agent, extract, metabolite, microorganism

INTRODUCTION

Ancient man is known to have utilized plants as drugs for millennia when confronted with illness and diseases (Sofowora 1993). The vast majority of modern medications were derived originally from ancient herbal traditions. Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value. There are numerous natural plant products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally (Heinrich *et al.* 2004). Herbal medicine in the simplest form is medicine or drug made fromplants and can so be said to possess several synonyms, all of which refers to plants as the raw materials for medicine and the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics has led researchers to investigate the antimicrobial activity of several medicinal plants (Penalver *et al.* 2005). Increasingly, the world is returning to natural remedies for 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.

In line with this thinking we have decided to investigate the phytochemical, proximate and *in vitro* antimicrobial properties of 10 medicinal plant seeds from Nigeria including *Albizzia lebbeck* (*Fabaceae*), *Strychnos spinosa* (*Loganiaceae*), *Myristica fragrans* (*Myristicaceae*), *Monodora myristica* (*Annonaceae*), *Aframomum melegueta* (*Zingiberaceae*), *Croton penduliflorus* (*Euphorbiaceae*), *Blighia sapida* (*Sapindaceae*), *Antiaris Africana* (*Moraceae*), *Thevetia nerifolia* (*Apocynaceae*) and *Terminalia catappa* (*Combretaceae*).

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 hrs. The extracts were concentrated after the extraction and preserved in the refrigerator at 4°C.

Phytochemical test

The different extracts were tested for the presence of chemical constituents such as saponins, anthraquinones, alkaloids, tannins, cardenolides and terpenes using standard methods described by Oderinde *et al.* (2008). Other constituents determined were:

Flavonoids: 0.5 g of 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 indicated the presence of flavonoids (Murugan and Kathaperumal 1987).

Phenols: 0.5 g of ethanolic extractwasfirst extracted with ethyl acetate and then the extracts were filtered. The development of a blue-black or brown coloration following the addition of ferric chloride indicated the presence of phenols (Trease and Evans 1983). This was also determined quantitatively using the method described by Swain (1979).

Phlobatannin: 0.5 g of the aqueous extract of the seed sample was 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 of the extracts (Henry 1993).

Chalcones: 2 ml of ammonia solution was added to 5 ml of the ethanol extract of each seed sample. Formation of a reddish color confirmed the presence of chalcones (Polk 1996).

Steroids: 2 ml of acetic anhydride was added to 0.50 g of each of the seed samples and cooled on ice. 1 ml of concentrated sulphuric acid was carefully added three times to obtain a color change

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Research Note

Table 1 Qualitative phytochemical screening of the aqueous extracts of 10 medicinal seeds.

Sample	AL	TA	SA	FL	AN	PHE	PH	CH	ST	TE	CAR	GL
Albizzia lebbeck	+++	+++	++	+	-	+++	++	+	++	++	+	+
Strychnos spinosa	+	+++	+++	-	++	++	++	-	+	-	++	++
Myristica fragrans	+++	++	+	++	-	+++	++	+	+	+	+	+
Monodora myristica	+++	++	-	++	-	++	+	+	+++	++	+	-
Aframomum melegueta	+++	++	++	+	++	+++	+	-	+	+	-	-
Croton penduliflorus	+++	++	-	++	-	+++	++	+	++	++	+	-
Blighia sapida	++	+	+	+++	-	+++	+	++	++	-	-	-
Antiaris africana	+++	+	+	++	-	++	++	+	+	+	+	+
Theveti anerifolia	+++	+	-	+	-	++	++	+	+	+	+	+
Terminalia catappa	++	+	+	-	+	++	+	-	+	-	-	-

+++ = 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 ten medicinal seeds (%).

Sample	Alkaloids	Tannins	Phenols	Phlobatannins	Steroids
Albizzia lebbeck	2.866	3.665	3.568	0.265	1.635
Strychnos spinosa	0.876	3.554	3.095	0.276	1.340
Myristica fragrans	2.428	2.709	3.469	0.240	1.615
Monodora myristica	1.880	2.771	2.754	0.240	1.635
Aframomum melegueta	2.684	2.796	3.498	0.223	1.492
Croton penduliflorus	2.319	2.808	3.366	0.292	1.555
Blighia sapida	1.424	2.659	3.282	0.224	1.541
Antiaris africana	1.734	2.547	2.952	0.297	1.357
Theveti anerifolia	1.826	2.572	2.768	0.216	1.467
Terminalia catappa	2.045	2.497	2.845	0.233	1.426

from violet to blue and blue to finally green. This color change confirmed the presence of steroids. Spectrophotometric determination was achieved using the method of Wall *et al*. (1952).

Glycosides: 0.5 g of each powdered sample was 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 indicated the presence of glycosides (Rahila *et al*. 1994).

Microorganisms and media

Microorganisms used in the present study were collected from The University College Hospital (UCH), University of Ibadan, Nigeria. These included: mold (*Aspergillus niger* and *Candida albicans*), Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Gentamicin (Sigma) and Tioconazole (Fluka) were used as the positive controls for bacteria and fungi, respectively (Oderinde *et al*. 2008). The microorganisms were cultured in Sabouraud dextrose agar (Le Ponte de Claix, France).

Antibacterial activity

Ethanol extracts of the seeds were used for the antimicrobial test. The agar-well diffusion method was used. The wells were marked and the prepared serial dilution of each sample was placed into the wells and allowed to diffuse properly for about 45 min. All bacterial plates were incubated for 24 hr at 37°C while the fungal plates were incubated for 48 hr at 28°C. A clear zone of inhibition was observed and the readings were taken accordingly. Different concentrations of the extracts were prepared which are 100.00, 50.00, 40.00, 30.00, 20.00, 10.00, 7.25, 7.00, 5.00 and 3.13% in order to determine the inhibitory concentration strength of each of the extracts (Dorothy 1992).

RESULTS AND DISCUSSION

Results of the qualitative phytochemical screening of the 10 studied medicinal plant seeds are presented in **Table 1**. Alkaloid, tannin, phenols, phlobatannins and steroids are present in all the seeds. Anthraquinone is absent in almost all the seeds studied except for *S. spinosa*, *A. melegueta* and *T. catappa*. Glycoside was found in trace amount in all the samples except in *S. spinosa* in moderate amount. None of these secondary metabolites were found in appreciable amounts in *T. catappa*. Alkaloids and phenol were the most predominant in the investigated plant seeds. These phytochemicals, particularly alkaloids, tannins, flavonoids and saponins show medicinal and 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 extracts being used as antimicrobial agents. **Table 2** presents the result of the quantitative phytochemical analysis of the extracts. This quantification is an estimation of the amount of these compounds in the extracts. This estimation showed that among all the compounds determined, tannin has the highest concentration of 3.66% in *A. lebbeck* while *T. nerifolia* has the least concentration of 0.22%. Phenol is the predominating compound in term of percentage concentration while phlobatannin was the least. The concentration of alkaloid ranged from 1.80 to 2.90%, steroid ranged from 1.300 to 1.70% with *A. lebbeck* also being the extract with the highest concentration. This high concentration of the alkaloid also reflects in the qualitative analysis as it was appreciably present (+++) in the extracts.

Table 3 Antimicrobial screening of the aqueous extracts of 10 medicinal seeds.

Samples	SA	BS	EC	PA	CA	AN	Control
Albizzia lebbeck	+	+	+	-	+	+	+
Strychnos spinosa	+	+	+	-	+	+	+
Myristica fragrans	+	+	+	+	+	+	+
Monodora myristica	+	+	+	-	+	+	+
Aframomum melegueta	+	+	+	+	+	+	+
Croton penduliflorus	+	+	+	-	+	+	+
Blighia sapida	+	+	+	+	+	+	+
Antiaris africana	+	+	+	+	+	+	+
Theveti anerifolia	+	+	+	-	+	+	+
Terminalia catappa	+	+	+	+	+	+	+

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

Table 4 Concentration strength of the aqueous extracts of 10 medicinal seeds.

Samples	SA	BS	EC	PA	CA	AN	Control
Albizzia lebbeck	12.50	12.50	25.00	NA	25.00	25.00	+
Strychnos spinosa	12.50	12.50	12.50	NA	25.00	25.00	+
Myristica fragrans	25.00	12.50	25.00	100.00	25.00	25.00	+
Monodora myristica	12.50	25.00	12.50	NA	25.00	25.00	+
Aframomum melegueta	12.50	12.50	12.50	100.00	25.00	25.00	+
Croton penduliflorus	12.50	12.50	25.00	NA	25.00	25.00	+
Blighia sapida	12.50	12.50	12.50	100.00	25.00	25.00	+
Antiaris africana	12.50	50.00	12.50	100.00	25.00	25.00	+
Theveti anerifolia	25.00	12.50	25.00	NA	25.00	25.00	+
Terminalia catappa	12.50	12.50	25.00	100.00	25.00	25.00	+

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

The *in vitro* study of the antimicrobial activity of the aqueous extracts of these plant seeds revealed that the extracts have activity against the tested organisms except for *Pseudomonas aeruginosa* in the case of *T. nerifolia*, *C. penduliflorus*, *M. myristica*, *S. spinosa* and *A. lebbeck*, as shown in **Table 3**. The activity of these extracts against this microorganism makes them a promising antimicrobial. Similarly, the extracts from the root, stem bark and leaves of *Blighia unijugata* Bak from Nigeria showed activity against some pathogenic organism (Oderinde *et al*. 2008). Also, these extracts from *B. unijugata* contain some phytochemicals found in the presently studied *Blighia* seeds.

From **Table 4** some of the extracts did not have activity against the growth of *P. aeruginosa*. From our previous study on the antimicrobial screening of the essential oil of some herbal plant from western Nigeria (Ajayi *et al*. 2008) we found that the activity of extracts were dependent on its composition. All the extracts were active against *C. albicans* and *A. niger* at a concentration of 25%. Most of the extracts were active against *B. subtilis* at 12.50% except *A. africana* at 50% and *M. myristica* at 25.50%. The highest sensitivity was shown against *B. subtilis* and the least against *P. aeruginosa*.

The antimicrobial activities of these extracts could be attributed to the presence of metabolites within them as shown in **Table 1**, especially the presence of metabolites like tannin, alkaloids, phenols, glycosides, anthraquinonse and flavonins (Chung *et al*. 1998).

CONCLUSION

A study was carried out on the phytochemical screening and antimicrobial activity of some seeds from Nigeria. This study suggests that these seeds could be explored as possible antimicrobial agents. However, further study could be carried out on the isolation of the precise bioactive compounds responsible for the activities exhibited by the extracts from these studied seeds.

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