Phytochemical Screening and Antimicrobial Activity of 10 Medicinal Seeds from Nigeria

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INTRODUCTION

Ancient man is known to have utilized plants as drugs for millennia when confronted with illness and diseases (Sofo-wora 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 from plants and can so be said to possess several synergistic properties of 10 medicinal plant seeds from Nigeria including Albizia 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), along with four drops of concentrated hydrochloric acid after which 0.5 g of magnesium turning was added. Development of pink of ferric chloride indicated the presence of phenols (Trease and Evans 1973). Spectrophotometry was used to determine the concentration of the extracts (Henry 1993).

Chalcones: 2 ml of ammonia solution 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.

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:

Phenols: 0.5 g of ethanolic extract was first 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).

Steroids: 2 ml of acetic anhydride was added to 0.50 g of each of the seed samples and concentrated. This was repeated two times to obtain a color change.

ABSTRACT

The phytochemical screening and antimicrobial activity of 10 medicinal seeds (Albizia 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

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.

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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 concentrated. This was repeated two times to obtain a color change.
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 (Rahtia 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 Claux, 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 (Sofo-wora 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 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 1 Quantitative phytochemical screening of the aqueous extracts of 10 medicinal seeds (%).

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

Table 2 Antimicrobial screening of the aqueous extracts of ten medicinal seeds.

<table>
<thead>
<tr>
<th>Samples</th>
<th>SA</th>
<th>BS</th>
<th>EC</th>
<th>PA</th>
<th>CA</th>
<th>AN</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albizia lebbeck</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Strychnos spinosa</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Myristica fragrans</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Monodora myristica</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aframomum melegueta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Bilghia sapida</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Antiaris africana</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thevetia anerifolia</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terminalia catappa</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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, anthraquinone 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.

**REFERENCES**


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

<table>
<thead>
<tr>
<th>Samples</th>
<th>SA</th>
<th>BS</th>
<th>EC</th>
<th>PA</th>
<th>CA</th>
<th>AN</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Albizzia lebbeck</strong></td>
<td>12.50</td>
<td>12.50</td>
<td>25.00</td>
<td>NA</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
<tr>
<td><strong>Strychnos spinosa</strong></td>
<td>12.50</td>
<td>12.50</td>
<td>12.50</td>
<td>NA</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
<tr>
<td><strong>Myristica fragrans</strong></td>
<td>25.00</td>
<td>12.50</td>
<td>25.00</td>
<td>100.00</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
<tr>
<td><strong>Monodora myristica</strong></td>
<td>12.50</td>
<td>25.00</td>
<td>12.50</td>
<td>NA</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
<tr>
<td><strong>Aframomum melegueta</strong></td>
<td>12.50</td>
<td>12.50</td>
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<td><strong>Crotol penduliflorus</strong></td>
<td>12.50</td>
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<td>12.50</td>
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<td>12.50</td>
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<td>25.00</td>
<td>25.00</td>
<td>+</td>
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<tr>
<td><strong>Antiaris africana</strong></td>
<td>12.50</td>
<td>50.00</td>
<td>12.50</td>
<td>100.00</td>
<td>25.00</td>
<td>25.00</td>
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<tr>
<td><strong>Thevetia arborilnia</strong></td>
<td>25.00</td>
<td>12.50</td>
<td>25.00</td>
<td>NA</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
<tr>
<td><strong>Terminalia catappa</strong></td>
<td>12.50</td>
<td>12.50</td>
<td>25.00</td>
<td>100.00</td>
<td>25.00</td>
<td>25.00</td>
<td>+</td>
</tr>
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**ACKNOWLEDGEMENTS**

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