

**ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF *Diospyros monbuttensis* (Fam: Ebenaceae) ROOT AND STEM BARKS.**

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**ABSTRACT**

Stem and root barks of *Diospyros monbuttensis*. Gurke are used in ethnomedicine for their antimicrobial activity. But only studies on the leaves and seeds are documented. The *in vitro* antimicrobial activity of crude extracts of root and stem barks of *D. monbuttensis* were evaluated against four reference bacteria (*S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*) and two reference fungi (*A. niger* and *C. albicans*). Gentamicin and tioconzole were used as control. Extraction was carried out using solvents of different polarities such as, petroleum spirit, chloroform, methanol and water. The parameters evaluated include phytochemical constituents, extraction yields and antimicrobial activity using seeded plate method. Phytochemical screening showed the presence of saponins, anthraquinones, glycosides and alkaloids while tannins was absent. The yield showed that methanolic and aqueous extracts were highest at 9-11%, and there was no significant difference ( $p>0.05$ ) between the percentage yields from the stem and root barks. The zones of inhibition obtained indicate that methanolic extracts had the highest activity against *Staphylococcus aureus* at 25 mg/ml and 50 mg/ml against *Psuedomonas aeruginosa* and 100 mg/ml against *Escherichia coli*, and *Bacillus subtilis* while the petroleum spirit and chloroform extracts had antifungal activity at 100 mg/ml. The crude extract of *D. monbuttensis* stem and root barks can be used for treatment of infections by susceptible organisms.

**KEY WORDS:** *Diospyros monbuttensis*, extraction, antimicrobial activity, root and stem bark.

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**INTRODUCTION**

*Diospyros* is one of the seven genera of the family Ebenaceae and is probably the only representative of this family in Nigeria. They are forest trees with hard,

heavy, strong and elastic wood. The colour varies from dirty white to pale brown and often times turns yellow or darkens on exposure. Only a few species produce a large amount of black heart wood<sup>(1)</sup>. There are about 26 documented

species in Africa most of which are also found in Nigeria<sup>(2)</sup>. They are commonly used as chewing sticks. Some of them have also been used in herbal medicines in the treatment of diseases such as leprosy, cough, as antiseptic washes for sores and wounds etc<sup>(3)</sup>. *Diospyros kaki* was the first of the *Diospyros* to be investigated<sup>(4)</sup> and since then extensive research has been carried out on this and other *Diospyros* species<sup>(1, 2, 3, 5, 6)</sup>. *Diospyros monbuttensis* leaves have been reported to possess antibacterial properties<sup>(5, 6, 7)</sup>. The antimicrobial activities of the plant leaves extract was due to the presence of tannins<sup>(2)</sup>. Other constituents that have been reported include saponins, anthraquinones, cardiac glycosides and alkaloids<sup>(6)</sup>. Aqueous and alcoholic extracts from the leaves of *Diospyros bateri* and *D. monbuttensis* were found to have strong antibacterial activity<sup>(2)</sup>, but no report on the root and stem barks have been documented. This work therefore is to study the antimicrobial properties of the root and stem barks which are also in use in herbal medical practice in Africa<sup>(5)</sup>.

## MATERIAL AND METHODS

### Materials

*Preparation of Plant material:* Root and stem barks of *Diospyros monbuttensis* were collected from University of Ibadan Botanical garden. Botanical identification and authentication was done by the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan, Nigeria, and samples were deposited in the same Institute. The plant parts were dried and pulverized.

*Microorganisms:* The microorganisms used include gram positive (*Staphylococcus aureus*, *Bacillus*

*subtilis*); Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and two reference fungi (*Aspergillus niger* and *Candida albicans*). They were obtained from the laboratory stock of the Department of Pharmaceutical microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria.

*Chemicals:* Chloroform, Petroleum spirit, Methanol, concentrated sulphuric acid were obtained from BDH Chemicals Ltd. Dragendroff, Mayer and Wagners' reagents, Nutrient agar (DIFCO) and Sabouraud dextrose agar (Oxoid Ltd) were of analytical grade. Tioconazole and gentamicin (Pfizer, Inc. New York, USA) are standard antifungal and broad spectrum antibacterial agents used as positive controls against which the activities of the extracts were compared.

*Extraction procedure:* The powdered root and stem samples (25 g) each was extracted using 200 ml of either chloroform or petroleum spirit or distilled water, or methanol in a soxhlet apparatus and the extracts were dried *in vacuo* by a rotary evaporator.

### Phytochemical screening

The freshly prepared methanol extract of the plants was qualitatively tested for the presence of secondary metabolites using standard procedures<sup>(8)</sup>.

### Antimicrobial activity

Extracts were reconstituted with 50% methanol in water to various concentrations for the determination of minimum inhibitory concentration (MIC). The concentrations used included 25, 50 and 100 mg/ml. Each concentration was screened for antimicrobial activity using

the seeded nutrient agar and sabouraud carpeted plates.

For each of the bacterial seeded plates, positive control was gentamicin at a concentration of 10 mg/ml while tioconazole was used for the fungal carpeted plates. Triplicate determinations of the above procedure were done.

## RESULTS

Phytochemical screening showed presence of alkaloids, saponins, cardiac glycosides and antraquinones while tannins was absent in the powdered stem and root barks of *D. monbuttensis*. Table 1 showed the percentage yield of crude extract of root and stem bark of *D. monbuttensis* when the different extraction solvents was used.

## DISCUSSION

Plants produce many organic compounds which have value in the treatment of various diseases. Phytochemical screening showed presence of alkaloids, saponins, cardiac glycosides and anthraquinones while tannins was absent in the powdered stem and root barks of *D. Monbuttensis*. These results are similar to that of the plant leaves obtained in previous studies except for the presence of tannins in the powdered leaves which were reported to be responsible for the observed antimicrobial activity<sup>(2)</sup>.

Extracts are concentrated preparation of vegetable oils or animal drugs which contained large quantities of extraneous matters obtained by removal of active principles from respective plant parts with suitable menstrual and evaporation of all or nearly all of the solvent to obtain the active principle in purest possible state<sup>(8)</sup>. In this study, four extracting solvents with

different polarities have been used. The extraction yield show that the methanol and aqueous extracts were 11 and 9 %, respectively, compared to the extracts of chloroform and petroleum spirit which were 5 to 6 %, respectively. It appears that the extracts of *D monbuttensis* were more soluble in solvents with higher dielectric constants and hence higher polarities. Furthermore, there was no significant difference ( $p>0.05$ ) in the yield of root and stem bark extractions, this suggests that any part of the plant bark could be used.

The antimicrobial test showed that all the test microorganisms were sensitive to the control drugs, gentamicin or tioconazole at the concentrations tested. However, the aqueous extracts did not show any antimicrobial activity at 25 mg/ml while the methanol extract had the highest zones of inhibition followed by chloroform spirit and finally petroleum spirit extracts (see table 2). The result of aqueous and methanolic extract was similar to that of previous studies obtained from the leave extracts<sup>(2)</sup>. As the concentration increased, there was a corresponding increase in antimicrobial activity. The aqueous extract of root bark was active against *B. subtilis* at 50 mg/ml, and both gram positive organisms at 100 mg/ml. Petroleum and chloroform spirit extracts showed antifungal activities at 100 mg/ml. This showed that the antifungal activity is both solubility and concentration dependent. It is probable that the plant constituents that were soluble in the organic phase represented by petroleum and chloroform spirit had antifungal activity. Furthermore, as concentrations increased, there was a corresponding increase in the zones of inhibitions. This reveals that the plant bark extracts may have antifungal activity as opposed to

earlier reports that *D. monbuttensis* extracts did not have antifungal activity<sup>(2)</sup>. The sensitivities of these test organisms to the crude extract of *D. monbuttensis* stem and root barks shows that it can be useful in treatment of diseases caused by these organisms<sup>(10)</sup>. The result obtained from the kinetics of bacterial activity is shown in Fig 1. The 100 mg/ml methanol extract of root bark of *D. monbuttensis* that was used on *Escherichia coli* reduced the bacterial population to 0.054% after 4 h of contact.

## CONCLUSION

This study has shown that the crude extract of *D. monbuttensis* stem and root barks can be used for treatment of infections by susceptible organisms, because of its sensitivity to the extracts concentration of 25 mg/ml or more of the various extract. There was a more profound activity on *S. aureus* than any other microorganism used in this study.

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**Table 1:** Percentage yield of crude extract of root and stem barks of *D. monbuttensis*

Extraction solvent	% yield	
	Root	Stem
Petroleum Spirit	5.2	6.2
Chloroform	6.4	6.8
Methanol	10.3	11.6
Water	9.0	9.2

**Table 2:** Zones of inhibition (mm) of test micro-organisms by stem and root bark crude extracts of *D. monbuttensis* at 25 mg/ml

ORGANISM	Stem bark						
	Petroleum Spirit Extract	Chloroform Extract	Methanol Extract	Gentamicin Control	Aqueous Extract	Tioconazole Control	Methanol control
<i>P. aeruginosa</i>	-	10	-	25	-	NT	-
<i>E. coli</i>	-	-	9	28	-	NT	-
<i>B. subtilis</i>	-	-	-	30	-	NT	-
<i>S. aureus</i>	10	9	11	29	-	NT	-
<i>A. niger</i>	-	-	-	NT	-	23	-
<i>C. albicans</i>	-	-	-	NT	-	25	-
	Root bark						
<i>P. aeruginosa</i>	-	10	11	27	-	NT	-
<i>E. coli</i>	-	9	-	30	-	NT	-
<i>B. subtilis</i>	-	-	9	30	-	NT	-
<i>S. aureus</i>	11	12	13	32	-	NT	-
<i>A. niger</i>	-	-	-	NT	-	20	-
<i>C. albicans</i>	-	-	-	NT	-	25	-

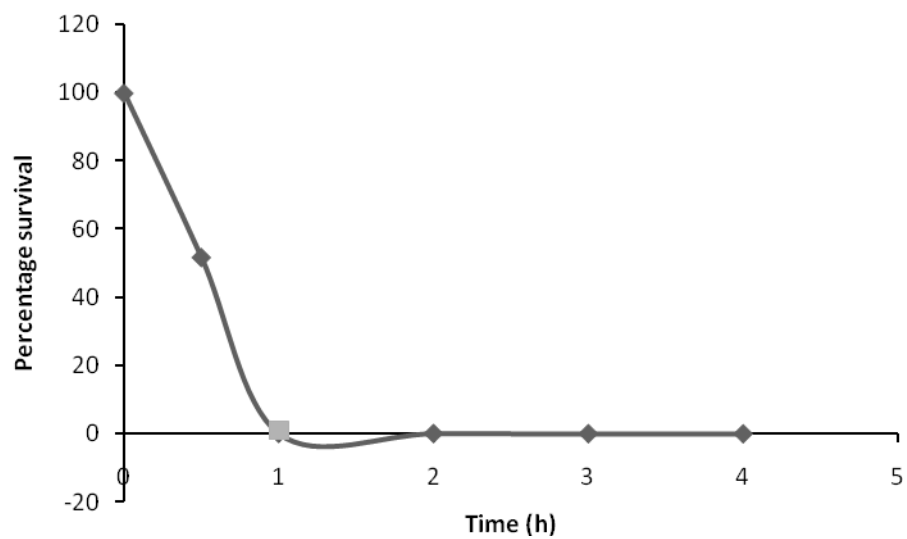
Key: - = no zone of inhibition    NT = not tested

**Table 3:** Zones of inhibition (mm) of test micro-organisms by root bark crude extracts of *D. monbuttensis* at 50 and 100 mg/ml, respectively.

ORGANISM	Root bark (50 mg/ml)						
	Petroleum Spirit Extract	Chloroform Extract	Methanol Extract	Gentamicin Control	Aqueous Extract	Tioconazole Control	Methanol control
<i>P. aeruginosa</i>	11	13	14	25	-	NT	-
<i>E. coli</i>	-	12	10	30	-	NT	-
<i>B. subtilis</i>	-	10	12	27	10	NT	-
<i>S. aureus</i>	13	15	16	22	-	NT	-
<i>A. niger</i>	-	-	-	NT	-	20	-
<i>C. albicans</i>	-	-	-	NT	-	25	-
	Root bark (100 mg/ml)						
<i>P. aeruginosa</i>	14	16	17	27	-	NT	-
<i>E. coli</i>	8	14	15	29	-	NT	-
<i>B. subtilis</i>	7	13	16	31	10	NT	-
<i>S. aureus</i>	16	17	19	28	9	NT	-
<i>A. niger</i>	-	10	-	NT	-	20	-
<i>C. albicans</i>	12	8	-	NT	-	25	-

Key: - = no zone of inhibition

NT = not tested



**Fig 1:** Kinetics of bactericidal activity of the methanol extract of root bark.