

Phytochemical and Antimicrobial Screening of the Leaf Extract of *Diospyros barteri*. Gurke.

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ABSTRACT

Diospyros bateri Gurke family Ebenaceae, has been commonly used by traditional medical practitioners as anti-infectious agent. In this study, preliminary screening of phytochemical constituents of *D. barteri* was carried out in addition to evaluating the antimicrobial activity of four different extracts of this leaf on some common bacteria species.

The antimicrobial activity of methanol, chloroform, hexane and aqueous extracts of the leaves were tested using the agar diffusion method on two Gram positive, three Gram negative bacteria viz; *Staphylococcus aureus*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*,

The preliminary phytochemical screening revealed the presence of alkaloids, saponins and tannins while other secondary metabolites were absent.

The study amply demonstrated the broad-spectrum antimicrobial property of the polar extracts while the non polar extract exhibits no activity. The minimum inhibitory concentration of the methanol extract on *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* were 1.20mg/ml, 2.24mg/ml, 3.55mg/ml, 9.12mg/ml and 10.0mg/ml respectively, while the minimum inhibitory concentration of the water extract on *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were 8.91mg/ml, 9.55mg/ml, 10.59mg/ml, 10.96mg/ml and 71.78mg/ml respectively.

Keywords: *Diospyros barteri*, antimicrobial, phytochemical screening

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INTRODUCTION

It is well known that misuse of antibiotics often leads to the appearance of resistant strains of micro-organisms^[1] and such misuse is becoming a norm in our present day society. In view of this propensity of bacteria to drug resistance, the search for new antibiotic continues unabated.

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins^[2].

Diospyros barteri belongs to the plant family, Ebenaceae. This is a family of trees and shrubs with alternate leaves which are always simple and have entire margins with no stipules. The fruit is a berry, surrounded at the base by the persistent and often enlarged calyx^[3]. *D. barteri* is a forest shrub or a small tree 3 to 22 feet high. It is sometimes scrambling, young plants are covered with ferruginous hairs. The leaves are glaucous (covered with white or bluish powder or bloom^[4], beneath. The fruits are pale yellow or sometimes orange.

The thin fruit-pulp is edible and is a minor item of diet^[5] the wood could also be used to make clubs, spear shafts, walking sticks and as house building materials^[3]. Ethno botanical uses in various part of West Africa includes control of diarrhea, dysentery, leprosy, antiseptic washes for sores and wounds, boiled leaves are applied as poultices for treating vaginal discharges^[6-7].

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A decoction of the leafy stems in drought is a common treatment for fever in Gambia^[8-9]. They are efficacious a chewing sticks in the Nigerian communities probably due to the presence of high fluoride (tannin) content in it^[10]

Biological investigation of the methanolic extract of two species of *D. barteri*, indicate that the seed extract was active against polio virus type 2 in the 'post-treatment assay.^[11]

This study is an attempt to determine the antimicrobial activity of the aqueous, hexane, chloroform and methanol extracts of *D. barteri*,

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *Diospyros barteri* was collected at Olokemeji, Abeokuta and authenticated by Mr. T. K. Odewo of the Forestry Research Institute of Nigeria (FRIN), where voucher specimen was deposited.

Plant preparation and extraction

The leaves of *Diospyros barteri* were ground (Hammer mill). Measured quantity of it was extracted respectively in hexane (1000g), methanol (400g), chloroform (500g) and distilled water (800g) by maceration at room temperature (30°C) for 72 hours respectively. The percentage yields of extracts in each solvent system were noted after removal of solvent.

Preliminary phytochemical screening

Air-dried and powdered plant materials were screened for the presence of tannins, alkaloids, anthraquinones, cyanogenetic glycosides, saponin glycosides, and steroidal nucleus flavonoids as described in literatures^[12-14].

Microorganisms

All the bacteria used were clinical strain obtained from the laboratory stock of the Department of Pathology, Olabisi Onabanjo University Teaching Hospital, Sagamu. Human pathogenic bacteria made up of 3 Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and 2 Gram-positive (*Staphylococcus aureus*, *Proteus vulgaris*) bacteria were used for the antibacterial assay,

Media

Nutrient broth, Nutrient agar, (LAB M™) were used in the study.

Antimicrobial Agents

Gentamicin, 2mg/ml, was used as the standard reference drug for antibacterial assays.

Preparation of bacterial cultures

The agar cup diffusion method was used to test the fractions for antimicrobial activity and determination of the minimum inhibitory concentration of the crude plant extracts, from stored slopes of bacteria, 5ml single strength nutrient broth was inoculated. The tubes were well shaken and incubated at 37°C for 18–24.

Using sterile pipettes, 0.2ml of 1 in 100 dilution of the bacterial cultures were added to 20ml of the melted and cooled (45–50°C) nutrient agar. The contents were mixed by gentle swirling movements before being poured into clean, sterile Petri dishes. After agar in plates has solidified, wells (6 mm each) were bored in each plate using an aseptic cork borer. Thereafter, wells were dug into the agar plates with the aid of sterile cork borer (6mm in diameter). Extracts were then reconstituted with appropriate solvents (chloroform, methanol, distilled water, hexane).

The concentrations used for the aqueous, methanol, chloroform and hexane extract of the leaf sample were; 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.625mg/ml, 7.8125mg/ml, 3.9063mg/ml, 1.953mg/ml.

The plates were then allowed to stand at room temperature for an hour to allow for adequate diffusion before incubating at 37°C for 24 hours for the bacteria.

The diameter of the zones of inhibition of the bacteria growth were measured to the nearest millimeter and recorded. The diameter of the zones of inhibition was measured along the two axes at 90 degree to each other and the mean of the two readings was calculated.

To determine the minimum inhibitory concentration MIC, the graph of the zone size (in mm) was plotted against the logarithm of concentration and the straight line obtained is extrapolated to appoint equivalent to the diameter of the cup bored. The antilog of the corresponding concentration obtained is the MIC^[14].

RESULTS

The percentage yield of the methanol, chloroform, hexane and distilled water extract of *D. barteri* was 26.8%, 8.2%, 6.5%, and 9.5% respectively as shown in Table 1

The results of the phytochemical screening indicated the presence of alkaloids, saponin glycosides, and tannins. For the antimicrobial activity, the diameters of the inhibition zones were measured in duplicate and the

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Table 1: Result of the phytochemical screening.

	<i>D. barteri</i>
Alkaloids	+++
Cardiac glycosides	----
Saponin glycosides	++
Anthraquinones	
Free	---
Combined	---
Cyanogenetic glycosides	---
Flavonoids	---
Tannins	+++

(-): Absent, (++): Fairly present, (+++): Abundant

result is presented as the mean of the two measurements as shown in Table 2. The MIC was determine graphically as a graph of zone of inhibition plotted and the log of concentration and the results is presented in Table 3

DISCUSSION

This study was designed to obtain preliminary information on the phytochemical constituents and antimicrobial activity of various extracts of *D.barteri* on five microorganisms. The cup plate diffusion method was used in this study.

Table 2: Result of the antimicrobial screening

Zone of inhibition in diameter

Test extract	Conc mg/ml	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
Methanol	1000	22.5	20.5	18.5	17.5	22.5
	500	20.2	18.5	18.0	16.8	20.0
	250	19.5	15.25	15.8	15.0	15.5
	125	18.0	15.0	14.25	15.0	15.0
	62.5	13.0	12.5	13.0	14.75	14.25
	31.25	9.5	9.5	11.0	12.5	13.5
	15.63	8.0	7.5	10.0	9.5	10.27
	7.81	----	----	8.75	7.5	9.5
	3.9	----	----	7.75	7.0	9.5
	1.95	----	----	----	----	----
Water	1000	26.5	20.5	16.5	24.5	24.8
	500	20.0	15.5	15.0	21.0	23.0
	250	13.0	12.75	13.0	18.75	17.5
	125	12.8	9.0	10.25	13.25	15.0
	62.5	12.5	----	10	13.25	15.0
	31.25	10.0	----	10.0	10.5	11.0
	15.63	9.0	----	8.0	9.5	10.28
	7.81	----	----	----	----	----
	3.9	----	----	----	----	----
	1.95	----	----	----	----	----
Gentamycin	2mg/ml	22.0	26.0	24.0	25.0	22.0

Table 3: Minimum inhibitory concentration

Test Organisms	MIC value in mg/ml	
	Water extract	Methanol extract
<i>Escherichia coli</i>	10.96	9.12
<i>Staphylococcus aureus</i>	71.78	10.00
<i>Proteus vulgaris</i>	9.55	1.20
<i>Pseudomonas aeruginosa</i>	10.59	2.24
<i>Klebsiella pneumoniae</i>	8.91	3.55

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The preliminary phytochemical screening revealed the presence of alkaloids, saponins and tannins while other secondary metabolites were absent. Earlier study indicated that the tannin present in the plant is hydrolysable tannin^[15]. The water and methanol extract of *D. barteri* were seen from the result to demonstrate broad antimicrobial action which are dose dependent.

There is an indication that the active antimicrobial constituent of the plant is a polar compound since the hexane and chloroform extract showed no activity at all on all the organisms.

In general, the methanol extract was found to have the best antimicrobial effect on the organisms, the solubility of active antimicrobial constituents of the extract in methanol must be greater than that of water considering the fact that they are both polar

The control experiment carried out using Gentamicin 2mg/ml gives the comparison of the activity of the extract and gentamicin [table 2]. The result shows that both the water extract and methanol extract at 1000mg and 500mg give activities that are quite comparable to that of gentamicin on most of the organism, also both extracts were able to inhibit *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Klebsiella Pneumoniae* at concentrations that are as low as 15mg/ml. this is an indication of the potency of this crude extract.

Test organisms used for this experiment have been implicated in diseases such as diarrhea, oral and dental infection, urinary tract infection, wound sepsis, dysentery and so on. These results therefore justify the local usage of this plant in the treatment of the various diseases. As earlier mentioned, the preliminary phytochemical screening revealed the presence of tannins, saponins and alkaloids. Tannin-containing drugs will precipitate protein; this could probably be responsible for the strong antibacterial activity exhibited by the plant^[16].

The efficacy of a given herbal drug in the treatment of an infectious disease relates to the presence of one or more

active agent in the herb that has antimicrobial activity of inhibiting the processes involved in the infection. There is therefore the need for bioactivity-guided fractionation and isolation of the active components in the plant extracts.

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