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IN-VITRO ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF DIOSPYROS MONBUTTENSIS

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ABSTRACT

Diospyros species in folklore medicine are used as anti-inflammatory, antibacterial, antioxidant, anticancer and antiviral agents. The *in vitro* antimicrobial activity of crude extracts of the leaves of *Diospyros monbuttensis* were evaluated against three bacterial species (*Staphylococcus aureus*, *Escherichia coli* and *Micrococcus luteus*) and fungal strain (*Aspergillus niger*). Extraction was carried out using both polar and non-polar solvents (ethanol and water). The leaves were screened for phytochemical constituents and preliminary screening for antimicrobial activity carried out using the agar well diffusion method. The minimum inhibitory concentration (MIC) was determined using the agar well dilution method. The phytochemical screening revealed the presence of saponins, tannins, glycosides and alkaloids in the plant. The ethanolic leaf extract of *D. monbuttensis* had no activity against the test organisms, but antimicrobial activity was observed for the aqueous extract against *S. aureus* and *E. coli* at all concentrations tested. The MIC of the aqueous extract of *D. monbuttensis* on *S. aureus* and *E. coli* was 0.78 mg/ml. The results of this study indicate that *Diospyros monbuttensis* leaves may be used for treatment of infections caused by *S. aureus* and *E. coli*.

Keywords: *Diospyros monbuttensis*; Antimicrobial activity; phytochemical screening

L'ACTIVITÉ ANTIMICROBIENNE IN VITRO D'EXTRAITS BRUTS DE DIOSPYROS MONBUTTENSIS

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RÉSUMÉ

Diospyros espèce dans la médecine folklorique sont utilisés comme anti-inflammatoire, Antibactérien, antioxydant, anticancéreux et d'agents antiviraux. L'activité antimicrobienne *in vitro* d'extraits bruts des feuilles de *Diospyros monbuttensis* ont été évaluées en fonction de trois espèces bactériennes (*Staphylococcus aureus*, *Escherichia coli* et *Micrococcus luteus*) et la souche fongique (*Aspergillus niger*). L'extraction a été réalisée en utilisant à la fois polar et les solvants non-polaires (éthanol et d'eau). Les feuilles ont été examinés pour constituants phytochimiques et contrôle préalable de l'activité antimicrobienne réalisée à l'aide de la méthode de diffusion et de l'agar. La concentration minimale inhibitrice (CMI) a été déterminé en utilisant la méthode de dilution en gélose bien. Le dépistage phytochimique a révélé la présence de saponines, tanins, glucosides cardiotoniques et d'alcaloïdes dans la plante. L'extrait de feuilles de éthanolique *D. monbuttensis* n'avait aucune activité contre les organismes à l'essai, mais l'activité antimicrobienne a été observée pour l'extrait aqueux contre *S. aureus* et *E. coli* à toutes les concentrations testées. Le MIC de l'extrait aqueux de *D. monbuttensis* sur *S. aureus* et *E. coli* était de 0,78 mg/ml. Les résultats de cette étude indiquent que *Diospyros monbuttensis* les feuilles peuvent être utilisées pour le traitement des infections causées par *S. aureus* et *E. coli*.

Mots-clés: *Diospyros monbuttensis* ; activité antimicrobienne ; dépistage phytochimiques

INTRODUCTION

Diospyros monbuttensis is commonly used as chewing sticks and also found varied uses in folklore medicine (1). It is widespread and can be found largely in West Africa and Asia. *Diospyros* species are used in folklore medicine as anti-inflammatory, antibacterial, antioxidant, anticancer, antiviral and termite resistant activities (2). *Diospyros monbuttensis* leaves have been reported to possess antibacterial properties (3). The antimicrobial activities of the plant leaves extract has been attributed to the presence of tannins, saponins, glycosides and alkaloids (3,4).

METHODS

Collection of Plant Materials: Leaves of *Diospyros*

monbuttensis were collected from Ota, Ogun state. The plants were identified in the Department of Pharmacognosy, Lagos University Teaching Hospital, Lagos state.

Plant Extraction

Plant materials for extraction were thoroughly washed and air dried at 37°C for 21 days and blended to powder using a laboratory blender (CentrifugeR (Model:CB8231-D) 1.75L). Ethanolic and aqueous extraction was carried out using the cold extraction method. The extracts were concentrated using a rotary evaporator and kept in tightly stoppered bottles in the refrigerator until the antimicrobial assay.

Microorganisms

The microorganisms used include Gram positive bacterial species (*Staphylococcus aureus*, *Micrococcus luteus*), Gram negative bacterial species (*Escherichia coli*) and one fungus (*Aspergillus niger*). They were obtained from the laboratory stock of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun state. The isolates were confirmed using conventional biochemical methods.

Determination of Antimicrobial Activity of Crude Extracts

Agar well diffusion method as earlier described with modification was used to carry out the antimicrobial activity testing of the crude extract (5). Three to five colonies of an overnight culture was transferred into test tubes containing 5ml of distilled water and adjusted to 0.5 McFarland standards. A sterile cotton swab was dipped into the adjusted suspension and used to streak the dried surface of Mueller Hinton agar plates (bacteria) and the potato dextrose agar plate (fungi). The inoculum was allowed to diffuse into the agar and a sterile cork-borer of 9mm diameter was used to bore uniform wells on the surface of the agar and after which the wells were filled with 0.4mls of the extract at concentrations of 100mgmL⁻¹, 75mgmL⁻¹, 50mgmL⁻¹ and 25mgmL⁻¹. The plates were incubated at 37 °C for 18-24 hours for bacteria. The fungal culture was kept at room temperature for 5 days after which the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (Mic)

Different concentrations of the extracts were prepared to give a final concentration in the range of 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.04875, 0.024375mgmL⁻¹. Two millilitre of each dilution of the extract was mixed with 18mL of molten Mueller Hinton agar, poured into petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. In all cases the lowest concentration at which there was no growth was recorded as the MIC.

Phytochemical Screening of Plant Samples

The freshly prepared aqueous extract of the plants was qualitatively tested for the presence of phytochemicals using standard procedures adapted from (6,7) [6,7].

RESULTS

The result for the antimicrobial activity of the ethanolic leaf extract of *Diospyros monbuttensis* was not active against *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* at all concentrations that were tested (Table 1). However, the aqueous extracts showed activity at 25mgmL⁻¹, 50mgmL⁻¹, 75mgmL⁻¹, 100mgmL⁻¹ against *S. aureus* and *E. coli* (Table 2). The minimum inhibitory concentration (MIC) of the aqueous extract of *D. monbuttensis* was 0.78mgmL⁻¹ for *S. aureus* and *E. coli*. The results for the phytochemical screening of the leaf of *Diospyros monbuttensis* as shown in Table 3 revealed the presence of saponins, tannins, glycosides and alkaloids.

TABLE 1: ANTIMICROBIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF *DIOSPYROS MONBUTTENSIS* AGAINST SELECTED MICROORGANISMS

Isolates	25mg/ml	50mg/ml	75mg/ml	100mg/ml
<i>Micrococcus luteus</i>	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-

Zone of Inhibition measured in millimetres (mm)

TABLE 2: ANTIMICROBIAL ACTIVITY OF AQUEOUS LEAF EXTRACTS OF *DIOSPYROS MONBUTTENSIS* AGAINST SELECTED MICROORGANISMS

Isolates	25mg/ml	50mg/ml	75mg/ml	100mg/ml
<i>Micrococcus luteus</i>	-	-	-	-
<i>Escherichia coli</i>	15	16	25	25
<i>Staphylococcus aureus</i>	11	12	17	19
<i>Aspergillus niger</i>	-	-	-	-

Zone of Inhibition measured in millimetres (mm)

TABLE 3: PHYTOCHEMICAL SCREENING OF *D. MONBUTTENSIS* LEAVES

Phytochemical tests	Result
Saponin	+
Flavonoids	-
Glycosides	+
Terpenoids	-
Tannins	+
Protein	-
Alkaloids	+

Keys: + Positive - Negative

DISCUSSION

The development of microbial resistance to the available antibiotics has informed the need to explore natural disease control options which has led to further investigation of antimicrobial activity of some medicinal plants (8,9). Studies have been carried out to discover useful antibacterial and antifungal compounds from plants (6,10). The results of this study showed that the ethanolic extracts of the plant leaves of *Diospyros monbuttensis* had no antimicrobial activities on the test organisms at all concentrations tested. The aqueous extracts of the plant showed promising antimicrobial activities. These results further validate the usefulness of this medicinal plant in traditional remedies among many tribes in the world in the treatment of bacterial infections.

D. monbuttensis is usually used as an antimalarial (3,11). The phytochemical screening of the plant revealed the presence of Saponins, Glycosides, Tannins and Alkaloids. The antimicrobial activities of this formulation may be due to its phytochemical

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constituents as earlier reported for some plants (12). Various studies have demonstrated that the alkaloids present in the leaves have a potential antioxidant property. This antioxidant activity is due to the ability to trap the free radicals and to chelate metal ions (2). Some analgesics and anti-inflammatory activities of this plant has been assigned to tannins. Apart from this, the tannins contribute to healing of wounds (13). Tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes.

Alkaloids and glycosides have a number of biological activities and strong antibacterial potentials. Saponins act by altering the permeability of cell walls and hence exert toxicity on all organized tissues. They exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (14). This property explain the mechanisms of antibacterial action of the aqueous extract of *Diospyros monbuttensis* against *Staphylococcus aureus* and *Escherichia coli*.

CONCLUSION

The results obtained from this study support the use of these plant parts in the traditional treatment of diseases in Nigeria. The results of this finding could be very important to pharmaceutical industries in the development of new antimicrobial drugs in order to address therapeutic needs. The screening of various natural organic compounds and identification of active agents may provide cheaper drugs that will be available and affordable to everyone. Further studies need to be conducted using different solvents, and there is also need for further studies on the plant parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

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