



PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL STUDIES OF THE STEM BARK EXTRACTS OF *DETARIUM MICROCARPUM* GUILL. & PERR (CAESALPINIACEAE)

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ABSTRACT

Detarium microcarpum is commonly known as “Tallow tree” and in Nigeria, it is referred to as “Taura” (Hausa), “Ogbogbo” (Yoruba), “Awakao fo” (Igbo) and widely distributed in semi-arid sub-Saharan Africa. Traditionally in Nigeria, *D. microcarpum* is used in the treatment of syphilis, fever, anaemia, stomach disorders. This study is aimed at evaluating the phytochemical constituents of *D. microcarpum* and the antimicrobial effects of the methanol and alkaloidal extracts on clinical isolates (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*) obtained from HIV patients. The phytochemical screening of *D. microcarpum* stem bark was conducted based on the method described in Trease and Evans’ pharmacognosy. The methanol and alkaloidal extracts of *D. microcarpum* stem bark was subjected to thin layer chromatography (TLC). The antimicrobial activity of the methanol and alkaloidal extracts of *D. microcarpum* stem bark at the concentrations of 0.125, 0.250, 0.500 and 1.000% was determined using cup-plate and tube-dilution method. The phytochemical screening revealed that *D. microcarpum* contains alkaloids, saponins, flavonoids, tannins, sterols and terpenes. Also, the TLC analysis showed that the alkaloidal extract contained several alkaloids with different R_f values. The zone of inhibition of the methanol extract of *D. microcarpum* ranged between 0.0 mm to 8.0 mm. Minimum Inhibitory Concentration (MIC) value of the methanol extract against *S. aureus*, *E. coli* and *S. typhi* were 1.25 mg/ml, 2.50 mg/ml and 2.50 mg/ml respectively, while the MIC value for the alkaloidal extract was 5.00 mg/ml against all the isolates. The stem bark of *D. microcarpum* contained phytochemical constituents, tannins, flavonoids, phenolic compounds, saponins and alkaloids, with antimicrobial activities which justify its use in traditional medicine against bacterial infection.

Keywords: Alkaloidal extract, Clinical isolates, *Detarium microcarpum*, Methanol extract, Phytochemistry,

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INTRODUCTION

Nature has been the ultimate source of raw material for medicinal agents since the history of man for the treatment of a wide spectrum of diseases [1]. An impressive number of modern Africans rely on plants and plant products for their medicines, with about 80% of African population relying on traditional medicine for their primary health care products [2]. Natural products and their derivatives accounted for about 75% of the anti-infective agents developed between 1981 and 2010 [3]. Microbial infection has been documented as a principal cause of death amid children, adolescents, and adults worldwide, and about 15 million (>25%) of the 57 million annual deaths worldwide are caused by infectious diseases [4]. Patient with advanced human immunodeficiency virus (HIV) infection are vulnerable

to bacterial diseases such as tuberculosis, bacterial pneumonia and septicaemia and malignancies often referred to as ‘opportunistic infections’ because they take advantage of the possibility offered by the undermined immune system. Antimicrobial drug resistance threatens successful prevention and treatment of infectious diseases [5]. The emergence of antibacterial resistance has led to the continuous search for new and effective antibacterial agents from natural sources for the treatment and prevention of diseases [6]. Alkaloids are a group of some naturally occurring basic chemical compounds that contain nitrogen atoms in one or more heterocyclic rings. They are produced by some plants and reptiles. Alkaloids have long been established to express potent biological activities in microbial infection and chronic diseases management [7]. *Detarium microcarpum* is a member of the family *Fabaceae* and is commonly referred to as “Sweet dattock” or “Tallow tree” and in Nigeria, it is referred to

as “Taura” (Hausa), “Ogbogbo” (Yoruba), “Awakao fo” (Igbo) and widely distributed in semi-arid sub-saharan Africa [8]. Traditionally in Nigeria, *D. microcarpum* is used in the treatment of syphilis, fever, anaemia and stomach disorders [9]. Several antibacterial compounds have been isolated from *Detarium species* such as *Detarium senegalense* by Donatus et al. [10]. This study is aimed at evaluating the phytochemical constituents of *D. microcarpum* and the antibacterial effects of the methanol and alkaloid extracts of *D. microcarpum* on some clinical isolates obtained from HIV patients.

MATERIALS AND METHODS

Preparation of plant material

The plant stem bark was collected in June 2020 from a wild source at Giwa Local Government Area of Kaduna state, Nigeria at about 10 am. The plant was identified and authenticated at the Herbarium section of the Department of Botany, Ahmadu Bello University, Zaria. The voucher number 900676 was issued. The stem bark was then air-dried and later pulverized.

Preparation of extracts

A stock of 750 g of powdered plant material was weighed and placed in a conical flask and macerated with redistilled methanol for 24 hours. The mixture was filtered and the marc was re-extracted successively with redistilled methanol until the extract became colourless. All the methanol extracts were pulled together and concentrated using rotary evaporator. The weight of the dried extract was noted and extract was stored in a refrigerator at -4°C until further use [11].

Phytochemical screening

The phytochemical screening of *D. microcarpum* stem bark was conducted in order to identify the various classes of phytochemical constituents using the standard methods of Trease and Evans pharmacognosy [11]. The extracts were screened for carbohydrates, tannins, flavonoids, anthraquinones, saponins, alkaloids, terpenes and steroids.

Extraction of alkaloids

The dried methanol extract (100 g) was dissolved in 100 mL of 5% HCl and filtered. The filtrate was partitioned with chloroform; the chloroform layer was collected and discarded (contain pigments and phenolic compounds), while the aqueous phase was made alkaline by addition of 10% ammonium hydroxide until the pH was at 11. The basified aqueous phase was further partitioned several times with chloroform and the chloroform fractions were pulled together and concentrated in the open air [11]. The weight of the alkaloid extract was noted.

Thin Layer Chromatographic (TLC) analysis of the crude alkaloid extract

The crude alkaloid extract was dissolved in methanol and spotted on the pre-coated silica gel TLC plate (thickness of silica gel: 0.25 cm) with the aid of a capillary-tube. The spot was allowed to dry before the TLC plate was placed in a tank saturated with chloroform / methanol (6:4). The plate was removed when the solvent front had travelled about three quarter of the plate, the solvent front was marked immediately before the plate was allowed to dry. The plate was then analyzed via:

Daylight visualization, Iodine vapour, Wagner’s reagent.

The same procedure as above was repeated for the alkaloid extract using chloroform / ethyl acetate (4:6) as the mobile phase.

Antimicrobial screening of *Detarium microcarpum* stem bark extracts

Test microorganisms

Clinical strains of Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative: *Escherichia coli* and *Salmonella typhi* were used in this study. All bacterial were obtained from HIV patient at Ahmadu Bello University Teaching Hospital, Shika, Zaria. They were re-evaluated at the Department of Pharmaceutical Microbiology laboratory, ABU, Zaria.

Preparation of cultures

An overnight bacteria culture was obtained by sub-culturing from the stored slopes into 5 mL nutrient broth and incubated for 18 hours at 37°C.

Preparation of plant extracts

Serial dilutions for each of the crude extract and were made using double dilution method (10 mg/mL – 1.25 mg/mL). They were used for the susceptibility testing of the extracts by agar diffusion technique.

Preparation of bacterial seeded agar plates

A 1 in 100 mL dilution of the overnight culture of each bacterium in appropriate medium was made by adding 0.1 mL into a test tube containing 9.9 mL of sterile distilled water. Using a sterile pipette, 0.2 ml of the 1 in 100 mL dilution of overnight culture of the test organism was seeded into 20 mL of melted and cooled agar medium and poured into sterile petri-dishes after thorough mixing between palms and allowed to set. Each plate was divided into four (4) portions using permanent marker and label T1, T2, T3, and T4 which correspond to 10 mg/mL, 5.0 mg/mL, 2.50 mg/mL and 1.25 mg/mL respectively. Equidistant wells were bored into the solidified medium using a sterilized cork borer of diameter 8 mm and each well was filled with 5mL of corresponding extract with the aid of a sterile syringe.

The plate was left at room temperature to pre-diffuse for 30 minutes and later incubated at 37°C for 24 h, after which the zones of inhibition were determined [12].

Determination of Minimum Inhibitory Concentration (MIC)

Thirty (30) sets of test tubes containing 5 mL of nutrient broth were prepared among which 3 contained double strength nutrient broth and the remaining 27 contained single strength. To each of the double strength broth was added 5 mL of the methanolic extract, mixed and from it 5 mL was withdrawn and transferred into the second test tube (single strength) and so on. From the test tube in the series, 10 test tubes were rejected. Each of the accepted test tubes in the series of 10 was then inoculated with 0.1 mL of the overnight broth culture of the test organisms which had been diluted to contain approximate bacterial count of 10^6 cfu/ml using a sterile inoculating loop. All the tubes were then incubated at 37°C for 24 h after which they were streaked on a sterile nutrient agar and incubated for 24 h. The MIC of the extract against each isolate was determined and noted. The same procedure was repeated with crude alkaloid extract [13].

RESULTS

The extractive value (% yield) of the methanol extract of the stem bark of *D. microcarpum* was 14.0% (105 g from 750 g powdered stem bark) while the percentage yield of the crude alkaloid extract (colourless crystalline powder) was 0.03% (0.03 g from 100 g of dried methanol extract). The phytochemical screenings of the methanol extract of *D. microcarpum* stem bark showed the presence of carbohydrates, reducing sugar, free anthraquinones, sterol/terpenes, flavonoids, saponins, tannins, hydrolysable tannins and alkaloids (Table 1). The TLC analysis of the crude methanol extract revealed ten (10) spots using chloroform: methanol (6:4) as the solvent system. The solvent front was 8.3 cm and the R_f value of the spots ranged from 0.048 – 0.867 (HR_f value: 4.8 – 86.7%) as shown in (Table 2). The TLC plate of the crude alkaloid extract was viewed after it was sprayed with Wagner's spray reagent; three (3) spots were revealed using chloroform: ethyl acetate (6:4) as the solvent system. The colours of the spots were; brown, light yellow and yellow with R_f value of 3.80, 6.30 and 27.5 respectively (Table 3). The bacterial susceptibility studies of the methanolic extract of *D. microcarpum* showed that the test organisms (overnight culture of the clinical isolates of *E. coil*, *S. typhi* and *S. aureus*) were susceptible to the extract at various concentrations. *E. coil* and *S. typhi* were susceptible to the extract at 5 mg/mL, whereas *S. aureus* was more susceptible to the extract at lower concentration of 1.25 mg/mL. The extract exhibited zones of inhibition ranging from 1.1 – 8.0 mm as shown in Table 4. The methanol extract of *D. microcarpum* has relatively uniform MIC for the bacterium cultures. *S. aureus* has the lowest MIC of 1.25 mg/ml, while *S. typhi* and *E. coil* have the same MIC of 2.50 mg/mL (Table 5). However, the crude alkaloid extract has a higher but uniform MIC of 5.0 mg/mL for the entire test organisms (Table 6).

Table 1: Phytochemical Screening of Methanolic Extract of *Detarium microcarpum*

| Constituent | Test | Observation | Inference |
|---|----------------------------|-----------------------------|-----------|
| Carbohydrate | Molisch's Test | Purple ring | + |
| Reducing Sugar | Fehling's Test | Brick-Red precipitate | + |
| Free anthraquinones | Borntrager's Test | Pink-Violet colour | + |
| Combined anthraquinones | Modified Borntrager's Test | Pink colour | + |
| Sterol / Terpenes | Salkowski Test | Reddish colour at interface | + |
| Flavonoids | Shinoda's Test | Pink precipitate | + |
| | NaOH Test | Yellow colour | + |
| Saponins | Frothing Test | Stable froth formed | + |
| Tannins | Lead subacetate Test | Brown precipitate | + |
| Hydrolysable Tannins Alkaloids | Ferric Chloride Test | Blue-Black precipitate | + |
| | Mayer's Test | White precipitate | + |
| | Wagner's Test | Whitish precipitate | + |
| | Dragendorff's Test | Red precipitate | + |

Key

+ = Present

- = Absent

Table 2: Summary of chromatographic results of methanol extract of *Detarium microcarpum*

| Spot s | Distance Travelled (cm) | Colour | | | R _f Value | HR _f Value (%) |
|-----------|----------------------------|----------------|------------------|-------------------|-------------------------|------------------------------|
| | | Day light | Iodine Vapour | Wagner's spray | | |
| 1 | 0.4 | Brown | Brown | Green | 0.048 | 4.80 |
| 2 | 0.6 | Grey | Brown | Pink | 0.072 | 7.20 |
| 3 | 1.1 | Pink | Pink | Brown | 0.133 | 13.30 |
| 4 | 1.6 | Green | Red | Brown | 0.193 | 19.30 |
| 5 | 2.2 | Light brown | Green | Green | 0.265 | 25.50 |

| | | | | | | |
|----|-----|--------------|-------------|--------------|-------|-------|
| 6 | 2.9 | Light green | Light green | Light yellow | 0.349 | 34.90 |
| 7 | 3.9 | Green | Yellow | Light yellow | 0.470 | 47.00 |
| 8 | 5.3 | Light yellow | Red | Light yellow | 0.639 | 63.90 |
| 9 | 6.2 | Brown | Deep red | Yellow | 0.747 | 74.70 |
| 10 | 7.2 | Yellow | Yellow | Green | 0.867 | 86.70 |

Mobile Phase: chloroform: methanol (6:4)
Solvent Front: 8.3 cm

Table 3: Summary of chromatographic results of crude alkaloid extract of *Detarium microcarpum*

| Spots | Distance Travelled (cm) | Colour | | | R _f Value | HR _f Value (%) |
|-------|-------------------------|--------------|---------------|----------------|----------------------|---------------------------|
| | | Day light | Iodine Vapour | Wagner's spray | | |
| 1 | 0.3 | Light yellow | Brown | Brown | 0.038 | 3.80 |
| 2 | 0.5 | Light yellow | Light yellow | Light yellow | 0.063 | 6.30 |
| 3 | 2.2 | Light yellow | Light yellow | Yellow | 0.275 | 27.5 |

Mobile Phase: chloroform: Ethyl acetate (4:6)
Solvent front: 8.0 cm

Table 4: Mean of zones of inhibition (mm) of *Detarium microcarpum* methanol extract on clinical bacteria isolates

| Dose of extract (mg/mL) | Mean Zones of Inhibition (mm) on test organisms | | |
|-------------------------|---|-------------------------|------------------------------|
| | <i>Escherichia coli</i> | <i>Salmonella typhi</i> | <i>Staphylococcus aureus</i> |
| 10 | 2.0 | 2.1 | 8.0 |
| 5 | 1.5 | 1.1 | 4.5 |
| 2.5 | 0.0 | 0.0 | 2.5 |
| 1.25 | 0.0 | 0.0 | 1.2 |
| Ciprofloxacin | 3.6 | 3.0 | 3.8 |

Table 5: Minimum Inhibitory Concentration (MIC) values of methanol extract of stem bark of *Detarium microcarpum* on test organisms

| Test Organisms | Dose of Extract (mg/mL) | | | | | | | | | MIC (mg/ml) |
|------------------------------|-------------------------|------|------|------|------|------|------|------|------|-------------|
| | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.17 | 0.04 | 0.02 | 0.01 | |
| <i>Staphylococcus aureus</i> | - | - | - | + | + | + | + | + | + | 1.25 |
| <i>Salmonella typhi</i> | - | - | + | + | + | + | + | + | + | 2.50 |
| <i>Escherichia coli</i> | - | - | + | + | + | + | + | + | + | 2.50 |

Table 6: Minimum Inhibitory Concentration (MIC) values of alkaloid extract of stem bark of *Detarium microcarpum* on test organisms

| Test organisms | Dose of Extract (mg/mL) | | | | | | | | | MIC (mg/mL) |
|------------------------------|-------------------------|------|------|------|------|------|------|------|------|-------------|
| | 5.00 | 2.50 | 1.25 | 0.63 | 0.31 | 0.17 | 0.04 | 0.02 | 0.01 | |
| <i>Staphylococcus aureus</i> | - | + | + | + | + | + | + | + | + | 5.00 |
| <i>Salmonella typhi</i> | - | + | + | + | + | + | + | + | + | 5.00 |
| <i>Escherichia coli</i> | - | + | + | + | + | + | + | + | + | 5.00 |

Key

- + = Growth
- = No growth

DISCUSSION

The percentage yield of methanol extract from the stem bark of *D. microcarpum* was 14.0%. The alkaloids present in the stem bark of *D. microcarpum* are however small and account for about 0.03% of the methanol extract of the stem bark. These extractive values can be used for quality analysis. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvents. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug. Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying, storage or formulation [11].

The result of the phytochemical screenings of the methanol extract of *D. microcarpum* stem bark indicated the presence of the following compounds:

carbohydrates, reducing sugar, free anthraquinones, sterol, terpenes, flavonoids, saponins, tannins, hydrolysable tannins and alkaloids. About 25 to 50% of current pharmaceuticals including antibacterial products have their origin from plants secondary metabolites, such as anthraquinones, sterol, terpenes, flavonoids, saponins, tannins, and alkaloids because they have been found to have active antimicrobial properties *in vitro* [11, 14].

From the result of the TLC analysis and phytochemical screening of the crude methanol extract, it was observed that the methanol extract contained several groups of phytochemical compounds (10 spots using chloroform: methanol (6:4) as the solvent system) than the alkaloids extract which has only three (3) spots using chloroform: ethyl acetate (6:4) as the solvent system. Wagner’s spray reagent indicated the presence of alkaloids. The polarity of alkaloids demands that moderately polar solvent system should be used to move and separate the alkaloids spots on a polar silica gel plate [11].

The result of the antibacterial studies of the methanol and alkaloid extracts of *D. microcarpum*

showed that *S. aureus* was more susceptible to the extract than *E. coli* and *S. typhi* in a concentration dependent manner similar susceptibility to the result obtained by Garba et al. [15]. Gram positive organisms are more susceptible than gram negative due to the difference in their cell wall composition. However, the methanol extract has higher antibacterial activity *in vitro* than the alkaloids extract, this may be attributed to the synergistic activities of the various phytochemicals present in the methanolic extract. There will be product development, structural elucidation and clinical studies.

CONCLUSION

The phytochemical screening of *D. microcarpum* stem bark revealed that it contained anthraquinones, sterols, terpenes, flavonoids, saponins, tannins, and alkaloids. The result of the antibacterial studies of *D. microcarpum* showed that the methanol extract has better activity than the alkaloid extract and this justifies the use of *D. microcarpum* traditionally in the treatment of infections such as fever and stomach disorders. The phytochemicals may be responsible for the antimicrobial activity.

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