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# Preliminary Phytochemical Evaluation and Antimicrobial studies of the methanol stem bark extract of *Detarium microcarpum.*

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## ABSTRACT

*Detarium microcarpum* is an African native plant that grows in the wild in several African countries, notably in savannah areas. The plant has a wide therapeutic application and is often referred to as the miracle plant by the traditional herbalist. Folk medicine relies heavily on its leaves and fruits. Hence, this research was aimed at qualitatively and quantitatively estimating the phytoconstituents inherent in the plant as well as to evaluate its antimicrobial potentials. Qualitative analysis of the methanol crude extracts was carried out to identify the presence of the classes of secondary metabolites: The results of the phytochemical screening revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids and Glycosides. Similarly, the quantitative estimation of these phytoconstituents revealed the following percentage of the metabolite; alkaloids (112.35 mg/100g Atropine sulfate eqv), saponins (96.84 mg/100g Gravimetric), Tannins (159.65 mg/100g Tannic acid eqv), Flavanoids (125.47 mg/100g Quarcetine eqv) Glycosides (397.53 mg/100g), Steroids (34.52 mg/100g Cholestrol), Total phenolics (228.24 mg/100 Gallic acid eqv). The paper disc diffusion method was used to determine the antimicrobial activity of the Crude methanol extract of *Datarium microcarpum* using standard procedures (Bauer *et al* 1996). The results of antimicrobial efficacies revealed that the crude methanol extract of stem bark *Detarium microcarpum* was effective in inhibiting the growth of the following microorganisms; *Protius*, *Bacillus subtilis staphylococaccus aureus*, *Enterobacta*, *Eschericia coli Shigelia dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aspergillus nigger*, *Aspergillus flavus*, *Candida albican and Rhezome*. The methanol crude extract showed a broad spectrum of antimicrobial activity with minimum inhibitory concentration (MIC) from $1.0\times10^{4}$ to $4.0\times10^{4}$.

**Keywords: Phytochemical, antimicrobial studies, stem bark, and *Detarium microcarpum***

## INTRODUCTION

In recent years, as infectious diseases have spread and harmful organisms have developed resistance to therapy, the quest for new treatments has increase substantially [1]. Humans have always drawn inspiration from plants due to their unique medical characteristics. Traditional African medicine relies heavily on the use of medicinal plants for the treatment of a wide variety of illnesses [2, 3]. Medicinal plants have been used by native peoples for centuries to cure and prevent illness, and they also serve as a source for a wide range of useful pharmaceuticals and other health aids [4]. Due to the rise in infectious diseases and treatment resistance among pathogenic organisms' the search for novel drugs has intensified significantly [5, 6]. Many of the chemical substances found in plants have physiological functions and can be used to treat or prevent disease [7]. Compounds that exhibit biological activity include flavonoids, tannins, saponins, alkaloids, glycosides and phenolics, these metabolites can be employed to treat or prevent diseases [8, 9]. *Detarium microcarpum* is an African native plant that grows in the wild in several African countries, notably in savannah areas. Because every part of the *D. microcarpum* plant has a therapeutic application, the plant itself is referred to be a miracle plant by the traditional herbalist. Folk medicine relies heavily on its leaves and fruits. The need for new drugs derived from numerous species of medicinal plants is continually growing today [10]. Hence, this research was aimed at qualitatively and quantitatively estimating the phytoconstituents

inherent in the plant as well as to evaluate its antimicrobial potentials.

## MATERIALS AND METHODS

The stem bark of *Detarium microcarpum* was collected from Dass LGA of Bauchi State, Nigeria. The plant material was identified and authenticated by a plant taxonomist in the Biological Science Gombe State University and Voucher Specimen Number GSU H369 was given to the plant. The sample was deposited in the research Laboratory of Chemistry Department Modibbo Adama University Yola. The solvent used for the preparation of the stem bark extract were methanol and n-butanol.

### Preparation of Plant Extract

The plant material was dried at room temperature and then grounded using mortar and pestle. The powdered sample (2.5 kg) was subjected to soxhlet extraction using methanol as solvent. The resulting extract was concentrated on a hot water bath and stored in desiccators for further investigation.

### Phytochemical screening

The concentrated crude methanol extract was subjected to phytochemical screening using standard i.e qualitative methods as described by [11] also supported by [12 – 20]; to identify the presence of the classes of secondary metabolites. The extract was screened for the presence of Alkaloids, Saponins, Tannins, Flavonoids, Steroids and Glycosides. Similarly, quantitative analysis was also deployed to estimate the amount of each secondary metabolite in the extracts.

### Test Organisms

Standard strains of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Candida albicans*, *Rhezome*, *Aspergillus nigger*, *Protius Bacillus*, *subtilis*, *Staphylococcus aureus*, *Enterobacter* were obtained from federal medical centre, Gombe as a clinical isolate, Gombe state Nigeria.

### Antimicrobial screening test

The paper disc diffusion method was used to determine the antimicrobial activity of the Crude methanol extract of *Datarium microcarpum* using standard procedures [21]. Solution of the extract varying concentrations, ranging from 100 µg/ml to 400 µg/ml was prepared. Nutrient agar was prepared, sterilize and used as growth medium for the microorganisms. 20 ml of sterilized medium was poured into each sterilized petri-dish. Covered and allowed to solidify. The Mueller- Hinton sensitivity agar plate was then seeded with the test microorganism by the spread the plant technique and was left for about 30 min to dry. The sterilized paper discs were soaked in the prepared solution of the extract with varying concentration and were dried at 50 $^{0}$C. The dried paper discs were then planted on the nutrient agar seeded with the microorganisms. They were incubated at 37 $^{0}$C for 24h after which they were inspected for zones of inhibition were measured and recorded in millimeter.

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) was determined using the nutrient broth dilution technique as described by [22]. The MIC value was determined for the microorganisms that were sensitive to the extracts under study. The extract was first diluted to the highest concentration (400 mg/ml) in 85% methanol in distilled water (v/v), and then a two-fold serial dilution of each extract was made to a concentration ranging from 6.25 to 50 mg/ml using nutrient broth (13 g/l). To the suspension, 5 ml of each extract concentration was added into nutrient broth and then 1.0 ml of standardized broth cultures containing 1.0 MIC $10^{7}$ CFU/ml was seeded into each test tube and then incubated at 35°C for 18-24 hrs. MIC is defined as the lowest concentration where no turbidity was observed in the test tubes.

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) was determined using the nutrient broth dilution technique as described by [22]. The MIC value was determined for the microorganisms that were sensitive to the extracts under study. The extract was first diluted to the highest concentration (0.015g/ml) in DMSO in distilled and then a two-fold serial dilution of extract was made to a concentration ranging from 100 µg/ml to 400 µg/ml using nutrient broth (13 g/l). To the suspension, 5 ml of each extract concentration was added into nutrient broth and then 1.0 ml of standardized broth cultures containing 1.0 MIC $10^{7}$ CFU/ml was seeded into each test tube and then incubated at 35°C for 18-24 hrs. MIC is defined as the lowest concentration where no turbidity was observed in the test tubes.

**Innoculation of the Plates and Application of the Extracts**

The agar plates NA (nutrient agar) and SDA (soubourand dextrose agar) were inoculated by spreading a small volume (0.05 ml to 0.10ml) of the liquid inoculums (sub-cultured nutrient broth) by means of a wire loop. A microbe was inoculated in each plate to the desired number of microorganism. A sterilized cork borer of 6mm in diameter was used to bore the disk for the N-butanol fraction and two control (Afloxacine and Ketoconozole) disks were used. The test was carried out by using stock concentrations of 400 µg/ml 300 µg/ml 200 µg/ml 100 µg/ml for the crude methanol extracts of stem bark of *Detarium microcarpum* prepared by dissolving 0.005g of the extract into 1 ml of DMSO. Sterile filter paper disks were impregnated with the extracts at varying concentration of 400, 300, 200, and 100 µg/ml respectively. The experiment was performed in triplicate. Plates were aerobically incubated at 37 °C for 23 holes for the bacteria and 37 °C for 2-3 days for fungi. At the end of the incubation period, diameter of zones of inhibition was measured by means of transparent meter rule and was recorded, based on clinical Laboratory Standard.

## RESULTS

**Table 1: Phytochemical components of Crude methanol extract of *Detarium microcarpum* Stem bark**

| Phytochemical | Observation | Amount (mg/100g) |
|---|---|---|
| Alkaloids | ++ | **112.35** |
| Saponins | + | **96.84 mg** |
| Tannins | +++ | **159.65** |
| Flavonoids | ++ | **125.47** |
| Anthraquinones | - | - |
| Steroids | + | **34.52** |
| Glycosides | ++ | **397.53** |
| Phenolics | ++ | **228.24** |

**KEY: *+ TRACE, ++ MODERATE, +++ EXCESS, - NEGATIVE***

**Table 2: Suscitivity Test of Crude Methanol/Zone of Inhibition on Gram Positive Organisms**

| MeOH **(µg/mL)** | E.COLI (mm) | SHE(mm) | SAL(mm) | PSE(mm) |
|---|---|---|---|---|
| 400 | 13 | 17 | 16 | 14 |
| 300 | 12 | 15 | 14 | 10 |
| 200 | 10 | 14 | 11 | 8 |
| 100 | 8 | 8 | 10 | 7 |
| Standard | 7 | 7 | 6 | 6 |

**KEY: MeOH- Methanol Fraction, *E.COLI- Escherichia coli, SHEG- Shigella dysenteriae, SAL- Salmonella typhi, PSEUDO- Pseudomonas aeruginosa***

**Table 3. Suscitivity Test of Crude Methanol Extract/Zone of Inhibition on Gram Negative Organisms**

| Methanol µg/ml) | P.R(mm) | B.A(mm) | S.A(mm) | E.N(mm) |
|---|---|---|---|---|
| 400 | 15 | 12 | 12 | 11 |
| 300 | 15 | 11 | 10 | 11 |
| 200 | 15 | 11 | 10 | 10 |
| 100 | 10 | 8 | 10 | 9 |
| STANDARD | 9 | 18 | 20 | 17 |

**KEY: *P.R- Protius, B.S- Bacillus subtilis, S.A- Staphylococcus aureus, E.N- Enterobacter,***

**Table 4: Sensitivity Test of Methanol Crude Extract / Zone Of Inhibition on Fungal Organisms**

| Methanol | A.F.F(mm) | C.A(mm) | RHZ(mm) | ANA(mm) |
|---|---|---|---|---|
| 400 µg/ml | 18 | 18 | 11 | 18 |
| 300 µg/ml | 14 | 9 | 8 | 16 |
| 200 µg/ml | 1 3 | 10 | 6 | 8 |
| 100 µg/ml | 10 | 8 | 6 | 6 |
| STANDARD | 12 | 10 | 8 | 6 |

**KEY:*A.F.F-Aspergillus flavus, C.A-Candida albicans, RHZ-Rhezome, ANA-Aspergillus niger***

## DISCUSSION

The qualitative phytochemical evaluation of the extracts revealed the presence of alkaloids, saponnis, tannins, flavonoids steroids and glycoside were detected in Crude methanol extracts. The quantification of these metabolites yielded the following percentages per metabolites; alkaloids (112.35 mg/100g Atropine sulfate eqv), saponins (96.84 mg/100g Gravimetric), Tannins (159.65 mg/100g Tannic acid eqv), Flavanoids (125.47 mg/100g Quarcetin eqv) Glycosides (397.53 mg/100g), Steroids (34.52 mg/100g Cholestrol), T.phenolics (228.24 mg/100 Gallic acid eqv). These metabolites are enough to elicit pharmacologica response as was reported by [23]. Similarly, the observed antimicrobial potentials of the extracts could be due to the presence of these phytochemical constituent. The result of the phytoconstituents evaluation is similar to the findings of [24]. The antimicrobial activity of the methanol extract of *D. microcarpum* extracts against *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas*, *aeruginosa Protius*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter*, *Aspergillus flavus* at different concentration were observed. The results revealed higher zones of inhibition were observed on fungal (*Aspergillus flavus*, *Candida albicans Rhezome*, *and Aspergillus nigger)*. According to [25], the antimicrobial activity exhibited *D. microcarpum* suggests the presence of growth inhibiting phytochemical such as flavonoids and tannins. The higher efficacy observed could be attributed to the mode of extraction and solvents selection. The methanol stem bark extract showed higher zones of inhibition against Gram positive organisms *Escherichia coli* with 12 mm, *Shigella dysenteriae* with 16.5 mm (400 µg/ml), at (300 µg/ml) *Escherichia coli* with 10 mm, *Shigella dysenteriae* with 14 mm, at (200 µg/ml) *Escherichia coli* with 9 mm, *Shigella dysenteriae* with 12 mm. at at (100 µg/ml) *Escherichia coli* with 8 mm, *Shigella dysenteriae* with 10 mm, all the extract were Nil on ***S****almonella typhi* and lowest zone of inhibition *pseudomonas*. On gram negative organism the extract shows the lowest zone of inhibition on *Protius*, *Bacillus subtilis Staphylococcus aureus*, *Enterobacter*, and resistance on *Protius*, and *Bacillus subtilis* at (100 µg/ml) and the extract shows the highest zone of inhibition at different concentration ranging from 100 µg/ml to 400µg/ml on all the fungal organisms. The larger zones of inhibition exhibited by the fungal organisms such as *Aspergillus flavus*, *Candida albicans*, *Rhezome*, *and Aspergillus nigger* may be due to presence of variety of active compounds in the plant such as alkaloids, saponnins tannins flavonoids, steroids and glycosides as described by [26].

Minimum inhibitory Concentration (MIC) is the lowest concentration of the antimicrobial agent required to inhibit microbial growth. Clinically, MIS is not only used to determine the amount of antibiotics the patients will receive but also the type of antibiotics used which will lower the opportunity for microbial resistance to specific antimicrobial agents [27]. In this study the minimum inhibitory concentration was observed by Rhezome and *Aspergillus nigger* on fungal organisms, *Staphylococcus aureus Enterobacter*, on gram negative organisms and *Pseudomonas aeruginosa* on gram positive organism. However, some fungal and bactericidal organisms were reported to developed resistance due to the resistant mechanism they possess and as well as the thin peptidogly layer which found in Gram-negative bacteria. This means that, even Gram-positive bacteria are mechanically strong, but appears, to proffer little resistance to the diffusion of antimicrobial molecules. *E. coli* on the other hand, a Gram-negative bacterium is surrounded by a second membrane, the outer membrane which functions as an effective barrier [28]. In this study the methanol stem bark extract of *D. microcarpum* showed bactericidal and fungal effects against the clinical isolates of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas*, *aeruginosa Protius*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter*, *Aspergillus flavus*. These indicate that the stem bark extract can inhibits the organisms.

## CONCLUSION

The phytochemical analysis of the methanol stem bark extract of *D. microcarpum* reveal the presence of bioactive components including Alkaloids, Saponins, Tannins, Flavonoids Anthraquinones Steroids and Glycosides. The methanol stem bark extract showed higher zones of inhibition against Gram positive organisms *Escherichia coli*, *Shigella dysenteriae*, *Escherichia coli*, *Shigella dysenteriae*, *Escherichia coli* and ***S****almonella typhi* and lowest zone of inhibition pseudomonas, *Staphylococcus aureus*, *Enterobacter*, and resistance on *Protius*, and Bacillus *subtilis*.

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