NIJBAS
Publications 2024

OPEN ACCESS
ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES (NIJBAS)

Volume 5 Issue 1 2024

https://doi.org/10.59298/NIJBAS/2024/5.1.9296118

Quantitative Phyto-evaluation and Antimicrobial studies of the n-butanol stem bark fraction of *Detarium microcarpum*

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ABSTRACT

This paper examines the phytochemical constituents and antimicrobial efficacies of n-butanol stem bark fraction of *Detarium microcarpum using an in vitro model. The results revealed that the* N-butanol fraction of *Detarium microcarpum* stems tested positive for the presence of Alkaloids, Saponins, Tannins, Flavonoids, Anthraquinones, steroids, and Glycosides. Similarly, the *in vitro* antimicrobial study also revealed that the n-butanol fraction has effectively inhibited the growth of test organism's pathogens (*Protius, Bacillus subtilis, staphylococaccus aureus, Enterobacta, Eschericia coli, Shigelia dysenteriae, Salmonella typhi, Pseudomonas aeruginosa, Aspergillus nigger. Aspergillus flavus, Candida albicans, Rhezome).* The minimum Inhibitory Concentration (MIC). Range from 1.0×10^{4} to $4.0\times10^{4.}$
Keywords: Phytochemical Analysis, Antimicrobial Activity, *Detarium microcarpum*, n-Butanol Fraction, Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize numerous chemical compounds of biological importance, these compounds often have several functions in the plant which include defense against insects, fungi, and herbivorous mammals among others. Tapsell et al., [1] reported that more than 12,000 compounds have been isolated from plant sources. Chemical compounds in plants manifest their effect on the human body through processes identical to those already well understood for the chemical compound in conventional drugs; thus, herbal medicine does not differ greatly from conventional drugs in terms of how they work. This enables herbal medicine to have beneficial pharmacology but also gives them the same potential as conventional pharmaceutical drugs [2]. The use of plants and other natural products to treat ailments is almost worldwide among residents of developing countries, and the purchase of herbs and herbal products was considered more affordable than purchasing expensive modern pharmaceuticals. Data obtained from the World Health Organization revealed that the majority of the populations of Asia and Africa use herbal medicine for some aspect of primary health care. Moreover, the consumption of herbs and herbal products is not only limited to Africa and Asia, studies in the United States and Europe have revealed the use of herbs is getting acceptability though still less common in clinical settings. *Detarium microcarpum* is an African tree belonging to the Fabaceae (legumes) [3]. It is a small tree or shrub growing up to 15 m tall but can reach 25 m in moist areas [3]. In terms of growth rate, the shoots of the trunk can reach a height of 1.5 m – 2 min 1 to 2 years and are much more vigorous than seedlings which on average grow to 0.6 m after 3 years and may reach 1.5 m in 4 years [4]. It flowers during the rainy season in northern Nigeria (July to September/November), but the main flowering period only lasts up to 8 days. It bears fruit from September –January/May and in November; the tree sheds its leaves and produces new leaves in March [4]. Infectious diseases are currently the World's leading causes of premature deaths, killing almost 50,000 people every day [5]. The control of these diseases has posed new challenges because of the emergence of multiple drug resistance among several pathogens to some of the antimicrobial drugs commonly used in the treatment of infectious diseases [6]. Accordingly, [7] reported that the problem is further compounded by the indiscriminate use of antibiotics. In addition to this problem, some antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression, allergic reactions, and even loss of hearing [8]. These reactions and resistance have triggered the interest of researchers in the search for new antimicrobial substances, with much attention centered on natural products with biologically active compounds from plant species used in traditional herbal medicine. Several findings have been presented and

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

published on the comprehensive investigations of plant extracts from different species, and many of the results revealed significant inhibition and bactericidal effects on many microorganisms. Similarly, [9] noted that plant-based antimicrobials represent a vast untapped source of antimicrobials with enormous potential. Moreover, Rojas et al., 2006 think that the best way to prevent antibiotic resistance of pathogenic species is to develop new compounds that are not based on existing synthetic antimicrobial agents. This proposition was similar to the suggestion of [10] that plants are very effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. This paper seeks to evaluate the compounds inherent in the plant *Detarium microcarpum* as well as to investigate its antimicrobial potential using an in vitro model.

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 the Chemistry Department Modibbo Adama University Yola.

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 extracts were concentrated in a hot water bath and kept for further investigation, and then the methanol extract was subjected to partitioning using chloroform, ethyl acetate, N-butanol, and water as solvent. The N-butanol fraction was subjected to quantitative phytochemical and antimicrobial studies.

Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative and quantitative methods as described by [11, 12] for the screening of metabolites such as Alkaloids, Saponins, Tannins, Flavonoids Anthraquinones Steroids, and Glycosides.

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*, and were obtained from the federal medical center, department of medical microbiology, Gombe state Nigeria.

Antimicrobial Screening Test

The paper disc diffusion method was used to determine the antimicrobial activity of the N-butanol fraction of *Datarium microcarpum* using standard procedures [13, 14]. A solution of the extract in varying concentrations, ranging from 100 µg/ml to 400 µg/ml was prepared. Nutrient agar was prepared, sterilized, and used as a 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 concentrations 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 measured and recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the nutrient broth dilution technique as described by [15]. 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 the 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 [15]. 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 the 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 the 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.

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Inoculation 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) using a wire loop. A microbe was inoculated in each plate to the desired number of microorganisms. 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 N-butanol fraction partition portions, prepared by dissolving 0.005g of the extract into 1 ml of DMSO. Sterile filter paper disks were impregnated with the extracts at varying concentrations 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, the diameter of zones of inhibition was measured using the transparent meter rule and was recorded, based on clinical Laboratory Standards.

Table 1: Phytochemical components of N-butanol fraction from methanol extracts of *Detarium microcarpum* Stem bark

Phytochemical Compounds	QUALITATIVE	QUANTITATIVE
Phenolics	++	572.89 mg/100
Alkaloids	++	175.26 mg/100g
Saponins	+	53.51mg/100g
Tannins	+++	386.78mg/100g
Flavonoids	++	162.39 mg/100g
Anthraquinones	+	-
Steroids	+	41.76 mg/100g
Glycosides	++	249.85mg/100g

KEYS: + TRACE, ++ MODERATE, +++ EXCESS

Table 2: Susceptivility Test of N-butanol Fraction/Zone of Inhibition on Gram Positive Organism

N-butanol(µg/ml)	E.COLI(mm)	SHEG(mm)	SAL(mm)	PSEUDO(mm)
400	12	16.5	NIL	11.5
300	10	14	NIL	9
200	9	12	NIL	8
100	8	10	NIL	7
STANDARD AFLOXACINE(30µg/ml)	2	10	2	12.5

KEYS: N. B- N-butanol, E. COLI- ***Escherichia coli,*** **SHEG-** ***Shigella dysenteriae,*** **SAL-** ***Salmonella typhi,*** **PSEUDO-** ***Pseudomonas aeruginosa***

Table 3: Susceptibility Test of N-butanol Fraction/Zone of Inhibition on Gram-Negative Organisms

NBUTANOL(µg/ml)	P.R (mm)	B.S (mm)	S.A(mm)	E.N(mm)
400	10	12	15	13
300	8	10	12	11
200	6	8	8	10
100	NIL	NIL	11	8
STANDARD	16	17	15	18

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

Table 4: Susceptibility Test of N- butanol Fraction/Zone of Inhibition on Fungal Organisms,

N-butano**(µg/ml)**	A.F.F(mm)	C.A(mm)	RHZ (mm)	ANA (mm)
400	18	16	11	18
300	14	10	8	16
200	13	9	6	8
100	10	8	6	6
Standard (200 mg)	12	10	8	6

KEYS: A.F.F -*Aspergillus flavus,* C.A- ***Candida albicans,*** **RHZ-** ***Rhezome,*** **ANA-** ***Aspergillus nigger***

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ONLINE ISSN: 2992-5797
PRINT ISSN: 2992-6122

DISCUSSION

The result of the phytochemical analysis revealed the presence of alkaloids, saponnis, tannins, flavonoids anthraquinones, steroids, and glycoside in N-butanol partition portion extracts. The pharmacological efficacies of plant extracts have been linked to the phytoconstituents inherent in them [16]. Hence, the observed antimicrobial properties of the extract could be due to the presence of this phytochemical constituent. Similar findings were reported by [17] that the extract of *D. microcarpum* stem bark tested positive for similar metabolites. Moreover, the antimicrobial activity of the N-butanol partition portion from 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 revealed that the partitioned portions inhibited the microbial growth. Higher zones of inhibition were observed in fungal organisms (*Aspergillus flavus*, *Candida albicans Rhezome*, *and Aspergillus nigger)*. Similarly, the antimicrobial activity displayed by *D. microcarpum* partitioned portion suggests the presence of growth-inhibiting phytochemicals such as flavonoids and tannins which have been shown to possess antimicrobial potentials as was reported by [18]. Though, traditional practitioners primarily use water for preparation plant extracts from organic solvents have been found to display better antimicrobial activity compared to aqueous extracts. More so, the 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 [19]. 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 organisms. However, some fungal organisms and bactericidal organisms are resistant to the effect of the n-butanol partitioned portion. This may likely occur due to the resistance of the microorganism to the mechanism of action of the portion or due to the microrganism's thin peptidogly layer which is found in Gram-negative bacteria. This means that even Gram-positive bacteria are mechanically strong, but appear, 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 [20]. In this study, the N-butanol partition portion of 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 inhibit the organisms.

CONCLUSION

The phytochemical analysis of the N-butanol partition portion of the methanol stem bark extract of *D. microcarpum reveals* the presence of bioactive components including alkaloids, saponins, tannins, flavonoids, anthraquinones, steroids, and glycosides respectively. The N-butanol partition portion of methanol stem bark extract showed higher zones of inhibition against Gram-positive organisms such as *Escherichia coli*, *Shigella dysenteriae*, inactive against *Salmonella typhi* and lowest zone of inhibition *pseudomonas.* While 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).

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CITE AS: M.H Shagal, D. Abubakar, I.I Nkafamiya and A. M. Fulata (2024).Quantitative Phyto-evaluation and Antimicrobial studies of the n-butanol stem bark fraction of *Detarium microcarpum.* NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES, *5* (1):92-96.
https://doi.org/10.59298/NIJBAS/2024/5.1.9296118