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Antimicrobial Activity of Ethanolic and Methanolic Extracts of *Borassus aethiopium* Initial Shoot on Multi-drug Resistant Bacteria and Dermatophytes

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Authors' contributions

This study was carried out in collaboration between all authors. Author CA conceived the study, did the design and coordination. Author MMS wrote the first draft of the manuscript and managed the literature searches. Author OAG managed the analyses of the study. All authors participated in the laboratory processes. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To examine the phytochemical properties and chemical profile of the methanol and ethanol extracts of the initial shoot of *Borassus aethiopium* and to evaluate its antimicrobial activity on selected multidrug-resistant pathogenic bacteria and dermatophytes.

Place and Duration of study: Sampling was done from Plateau State, Nigeria, while the analysis was done at the Federal College of Forestry and National Veterinary Research Institute Vom, Plateau State Nigeria between August and December 2017.

Methodology: The chemical profile of *B. aethiopium* was determined using Gas Chromatography-Mass Spectrometry (GC-MS). Phytochemical analysis was performed to confirm the bioactive components present while the antimicrobial activity was determined using agar well diffusion method using *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Proteus*

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mirabilis, Trichophyton mentagrophytes and Epidermophyton floccosum as the test organisms. Minimum Inhibitory Concentration (MIC) was determined.

Results: All test organisms were susceptible to both the extracts of *B. aethiopium. Klebsiella pneumoniae* was found to be most susceptible (33 mm) in the methanolic extract and *Staphylococcus aureus* as the most susceptible (31mm) in the ethanolic extract while *Epidermophyton floccosum* was the least susceptible of both the extracts (19mm; 20mm). Methanolic extract of *B. aethiopium* showed the lowest MIC on the test organisms compared to the ethanolic extract. The difference in the chemical composition of the extracts obtained was Tridecanoic acid, Valeric acid, 14-Octadecenoic acid, Heneicosanic acid, Erucic acid, and Oxacyclotetradecane for the methanol extract and Dodecanoic acid, Undecanoic acid, Docosanoic acid, Oleic acid, 9-Octadecenoic acid (Z)- and 9,12,15-Octadecatrienoic acid for the ethanol extract. Bioactive components present in *B. aethiopium* extracts (%) were saponins 14.40; 6.45, flavonoids 24.20; 12.40, cardiac glycosides 13.55; 8.20, steroids 6.11; 4.12, alkaloids 5.00; 2.00, terpenoid 12.50; 11.80 and tannins 1.20; 0.05.

Conclusion: The initial shoot of *B. aethiopium* has the potential as a source of antimicrobial essential to the pharmaceutical industries. However, the toxicological analysis is recommended to assess the toxicity and safety on sensitive organs of the animals.

Keywords: Ethanolic extract; Methanolic extract; Borassus aethiopium; MDR bacteria; dermatophytes.

1. INTRODUCTION

Antibiotic resistance was once believed to be associated with only hospitals and other health-care facilities, but a number of community factors are now known to be the major cause of antibiotic resistance. Also community-associated resistant strains have now been identified as the cause of many hospital-acquired infections [1]. Due to the normal genetic variation in bacteria and fungi populations, the individual organism may carry mutations that render antibiotics and antifungal agents ineffective, thereby conveying a survival advantage to the mutated strain. One way to prevent this resistance is by using new compounds not based on the existing synthetic antimicrobial agents [2]. Phytochemicals from medicinal plants that show antimicrobial activities have the potentials of filling this gap because their structures are different from those of microbial sources and their mode of action may likely differ [3].

Borassus aethiopium is a popular medicinal plant among the inhabitants in tropical African countries. The tree has a wide natural distribution in tropical Africa, Asia and New Guinea. This genus has six species of fan palms native to these countries. The tree is commonly called as African palmyra palm and is widely distributed in the tropical African regions. The chief product of the palmyra palm is the sweet sap obtained by tapping the tip of the inflorescence as is done with other sugar palms and to a laser extent with coconut [4]. The initial shoot of the palm is called “Murichi” in Hausa Language and is used by the local people of Northern Nigeria as a food source and has medicinal properties that give relief to some health challenges. The tree sap is taken as a laxative and medicinal values of the plants have been ascribed to its other parts [4]. The initial shoot of the plant is reported to have antimicrobial activities against bacteria [5,6] and fungi [7]. An infusion of the root is used to treat different ailment such as stomach ache, throat infection, bronchitis and syphilis [8]

Herbal medicine has become an integral part of the primary health care system of many nations [9,10]. According to World Health Organization report [11], nearly 70-80% of the world population depends on herbal medicine while majority of the total population in developing countries still use traditional folk medicine obtained from plant sources [12,13]. The demand for herbal remedies in developed and developing countries is on the increase [14]. The expensive modern medicine used for treating various infections and the acquisition of drug resistance particularly in the third world countries has necessitated the search for new and alternative effective agents from natural products [15]. Many studies conducted in Ethiopia have shown the antimicrobial activities of many indigenous plants used in traditional medicine [16,17].

Although antimicrobial agents have greatly reduced the threats posed by infectious diseases since their discovery in the 1940s [18], the recent rise in multidrug resistance in bacteria and fungi is seriously jeopardising the strides already made. The increased usage of antimicrobials to treat infections among other factors has led to the emergence of multi-drug resistance strains [19]. Such strains are now resistant to the first line of treatments and also to the more expensive second and third line antibiotics. The cost of treating infectious diseases is also worrisome [20] because of the number of patients in the hospitals and the duration of their stay in such hospitals with financial burden and treatment failure leading to high morbidity and mortality rates.

This study was therefore initiated to identify the bioactive constituents, evaluate the antimicrobial activity and determine the chemical structures of the ethanolic and methanolic extracts of the initial shoot of *B. aethiopium* which will consequently lead to the development of new antimicrobial agent with higher efficacy in combating health-threatening diseases caused by pathogenic resistant bacteria and dermatophytes. It will add to the list of medicinal plants native to Africa and Nigeria with curative properties which can be harnessed and also provide the pharmaceutical industries with vital data on the plant as a source of alternative antimicrobial agent.

2. MATERIALS AND METHODS

2.1 Sample Collection

Initial shoots of *B. aethiopium* was purchased from the local farmers in Riyom LGA of Plateau state and the identity of the plant material was authenticated at the Federal College of Forestry Jos while the test organisms; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Proteus mirabilis, Trichophyton mentagrophytes* and *Epidermophyton floccosum* were isolated from human wounds, ear swabs and urine for bacteria and from human scalps and skin scrapings for dermatophytes at the National Veterinary Research Institute Vom where their resistance to multiple antibiotics had already been established through antibiotic susceptibility test. The identity of each organism was confirmed using standard cultural, morphological and biochemical techniques [21,22] for bacteria and [23] fungal isolates.

2.2 Sample Processing and Analysis

The plant shoots were air dried, pulverised and extraction from the dried samples was carried out using the Soxhlet extraction method with methanol and ethanol as the solvents [24,25]. The extracts were then subjected to qualitative and quantitative phytochemical analysis to confirm the presence or absence of the bioactive chemical constituent using standard procedures [26,27,28,29]. Gas chromatography-Mass spectrometry (GC-MS) model QP210 plus Shimadzu, Japan was used to determine the chemical profile of the extracts. Agar well diffusion method carried out in duplicates was used to determine the susceptibility of the test organisms to the plant extracts [30]. Nutrient agar media was used. *Staphylococcus aureus* (ATCC 12600), *Pseudomonas aeruginosa* (ATCC 27853) and *Trichophyton mentagrophytes* (ATCC 34551) reference strains were used as controls while laboratory confirmed isolates of *Klebsiella pneumoniae*, *Proteus mirabilis,* and *Epidermophyton floccosum* were also used as controls. The potency of the extracts was measured by the clear zones of inhibition. Minimum Inhibitory Concentration (MIC) of the extract against the test organisms was carried out using nutrient broth with dilution at concentrations of 125, 62.5, 31.5, 15.63, 7.81, 3.91, 1.95 and 0.98 mg/ml and was determined by the lowest concentration of the extracts which showed no turbidity or inhibited the test organisms [31,32,33].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of *Borassus aethiopium*

The result of this study revealed that the bioactive components were present in both methanolic and ethanolic extracts of *B. aethiopium* as saponins, flavonoids, cardiac glycosides, steroid, alkaloid, tannins and terpenoids with flavonoid having the highest amount of 24.20% and 12.40% in the methanolic and ethanolic extracts, respectively while tannin had the least amount of 1.20% and 0.05% in both extracts, respectively (Table 1). This result is in agreement with similar studies [34,35] which reported the presence of tannins, steroids, flavonoids and saponins in the root and leaf extracts of the plant. These bioactive components have also been reported to have an antimicrobial effect [36,37,38,39]. Flavonoids which had the highest percentage occurrence protect against inflammation, free radicals, allergies, microbes, ulcers, platelets aggregation, tumours and hepatotoxins [40]. These findings, therefore, supports the use of *Borassus aethiopium* initial shoot as a traditional herbal remedy.

Table 1. Phytochemical screening of methanolic and ethanolic extracts of *Borassus aethiopium* Initial shoot

Constituents	Methanolic extracts (%)	Ethanolic extracts (%)
Saponins	14.40	6.45
Flavonoids	24.20	12.40
Cardiac glycosides	13.55	8.20
Steroids	6.11	4.12
Alkaloids	5.00	2.00
Terpenoids	17.50	11.80
Tannins	1.20	0.05

3.2 Chemical Profile of Methanol and Ethanol Extracts of *Borassus aethiopium* Initial Shoot

The GC-MS revealed a total of seventeen (17) chemical compounds from the extracts combined, with Tridecanoic acid, Valeric acid, 14-Octadecenoic acid, Heneicosanic acid, Erucic acid, and Oxacyclotetradecane, being specific to methanolic extract while Dodecanoic acid, Undecanoic acid, Docosanoic acid, Oleic acid, 9-Octadecenoic acid and 9,12,15-Octadecatrienoic acid were specific to ethanolic extract (Table 2). Similar study Agouru et al. [13] also reported an array of these chemical structures present in plants native to Nigeria. Their presence in *B. aethiopium* indicates the potential of the plant to produce new antimicrobial agents.

3.3 Antimicrobial Activities of Methanol and Ethanol Extracts of *B. aethiopium* Initial Shoot

Results of the antimicrobial activities of the methanolic and ethanolic extracts revealed that all test organisms were susceptible to the extracts of *B. aethiopium.* The ethanolic extracts exhibited less antimicrobial activity compared to the methanolic extract. *Klebsiella pneumoniae*was most susceptible (33 mm) to the methanolic extract while *Staphylococcus aureus* was most susceptible (31mm) to the ethanolic extract. *Epidermophyton floccosum* was the least susceptible of both the extracts at 19 mm and 20 mm (Table 3). Similar studies [41,35] confirm a reduced antimicrobial activity with ethanolic extracts against microorganisms which

Table 2. Chemical composition of methanol and ethanol extracts of *B. aethiopium* initial shoot

Name	Mol. Weight	Formula	Extracts	
			Methanol	Ethanol
Methyl tetradecanoate	242	$C_{15}H_{30}O_2$	+	+
9,12-Octadecadienoic	294	$C_{19}H_{34}O_2$	+	+
Octadecanoic acid	624	$C_{39}H_{76}O_2$	+	+
Pentadecanoic acid	316	$C_{18}H_{36}O_4$	+	+
9-Octadecanoic acid	440	$C_{25}H_{44}O_6$	+	+
Hexadecanoic acid	691	$C_{37}H_{74}NO_8P$	+	+
Tridecanoic acid	228	$C_{14}H_{28}O_2$	+	-
Valeric acid	312	$C_{20}H_{40}O_2$	+	-
14-Octadecenoic acid	296	$C_{19}H_{36}O_2$	+	-
Heneicosanic acid	340	$C_{22}H_{44}O_2$	+	-
Erucic acid	338	$C_{22}H_{42}O_2$	+	-
Oxacyclotetradecane	240	$C_{14}H_{24}O_3$	+	-
9,12,15-Octadecatrienoic acid	436	$C_{25}H_{40}O_6$	-	+
Dodecanoic acid	214	$C_{13}H_{26}O_2$	-	+
Undecanoic acid	186	$C_{11}H_{22}O_2$	-	+
Docosanoic acid	354	$C_{23}H_{46}O_2$	-	+
Oleic acid	340	$C_{21}H_{40}O_3$	-	+

Key: *+ = Present in the extract; = Absent in the extract*

Table 3. Antimicrobial activity of methanolic and ethanolic extract of *Borassus aethiopium*

Test organisms	Extract	Zone of inhibition (mm)				
		500 mg/ml	250 mg/ml	200 mg/ml	125 mg/ml	100 mg/ml
Staphylococcus aureus	M	32	29	25	20	16
	E	31	28	26	23	16
Pseudomonas aeruginosa	M	23	20	18	15	8
	E	22	20	17	15	12
Klebsiella pneumoniae	M	33	30	28	25	20
	E	28	24	21	16	14
Proteus mirabilis	M	30	27	25	21	18
	E	24	20	12	9	0
Trichophyton mentagrophytes	M	25	21	17	12	8
	E	22	18	15	12	8
Epidermophyton floccosum	M	19	15	11	9	7
	E	20	18	11	18	6

***KEY:** M = Methanolic Extract; E = Ethanolic Extract*

Table 4. Minimum inhibitory concentration (MIC) of methanolic and ethanolic extracts of *Borassus aethiopium*

Test organisms	Methanolic extract (mg/ml)	Ethanolic extract (mg/ml)
Staphylococcus aureus	15.63	31.25
Pseudomonas aeruginosa	31.25	62.5
Klebsiella pneumoniae	31.25	15.6
Proteus mirabilis	7.81	31.25
Trichophyton mentagrophytes	31.25	62.5
Epidermophyton floccosum	15.63	15.63

could be attributed to the incomplete solubility of the bioactive compounds in ethanol or the presence of some possible inhibitors. The sensitivity of microorganisms to antimicrobial agents is dependent on the cell wall permeability of antimicrobial agents and the sensitivity of the test organisms to the methanolic and ethanolic extracts. Therefore, it suggests that the extracts have permeability over the cell wall of these organisms which serve as a barrier to the most commonly used antimicrobial agents. The extracts showed a broad spectrum of activity as their activities were independent of Gram reaction with significant degrees of sensitivity. It was also observed that the methanolic extract generally showed the lowest MIC compared to the ethanolic extract (Table 4). The highest concentration of inhibition was at 62.5 mg/ml on *Pseudomonas aeruginosa and Trichophyton mentagrophytes* for the ethanolic extract while the lowest was at 7.81 mg/ml on *Proteus mirabilis* for the methanolic extract.

4. CONCLUSION

This study has shown that the initial shoot of *B. aethiopium* is one of the plants whose curative potentials may be harnessed to manufacture useful drugs that can be used as alternatives to the present drugs that are being resisted by the microorganisms. The study has also revealed that the methanolic and ethanolic extracts of the initial shoot have broad-spectrum antimicrobial activity and this implies that the extract possesses antibacterial and antifungal properties. The toxicological analysis is recommended to assess the toxicity and safety on sensitive organs of the animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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