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Borassus aethiopum Fruit Pulp Extract has Antimicrobial Activity on Selected Clinical Microbial Strains

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EA and NPA were involved in the study design, data collection, data analysis and the first draft of the manuscript. Authors DBK and MAT were involved in the study concept development, implementation and manuscript review. Authors FCMR and CL were involved in the study implementation, data interpretation and review of the manuscript draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: Antimicrobials of plant origin have a huge therapeutic prospective and can efficiently be used to treat infectious diseases with reduced or no side effects as related to using synthetic antimicrobials. A potential plant-based antimicrobial is that obtained from *Borassus aethiopum*. The anti-inflammatory, pro-apoptotic, antipyretic and anti-venom properties of extracts of *B. aethiopum* plant have been reported in literature these past few years.

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Objective: The antimicrobial property and phytoconstituents of aqueous ripe fruit extract of *B. aethiopum* were investigated.
Methods: The ripe fruit extract of *B. aethiopum* was prepared by macerating the mesocarps of the fruits and then screened for the presence of phytochemicals using standard methods. The extract's antimicrobial activity was studied by agar well diffusion method against *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 33495, *Proteus mirabilis* ATCC 25923, *Pseudomonas* aeruginosa ATCC 27853, *Staphylococcus saprophyticus* ATCC 15305 and *Staphylococcus aureus* ATCC 25923. Chloramphenicol mixed with tetracycline was used as a standard antibacterial agent (Positive control). Sterile distilled water was used as diluent for reconstituting the aqueous extract.
Results: The aqueous extract revealed the presence of tannins, terpenoids, saponins and cardiac glycosides. The antibacterial activity revealed that at 30%, 40% and 50% w/v (0.3g/ml, 0.4g/ml and 0.5g/ml) of the extract, all the test bacterial strains were susceptible to the aqueous ripe fruit extracts of *B. aethiopum*.
Conclusion: Ripe fruit extracts of *B. aethiopum* was rich in phytochemicals and exhibited potential antibacterial activity against all seven bacterial strains used.

Keywords: Borassus aethiopum; Arecaceae; antimicrobials; antibacterial; phytoconstituents.

1. INTRODUCTION

An immensely unexploited source of medicine is represented by plant-based antimicrobials. Antimicrobials of plant origin have a huge therapeutic prospective and can efficiently be used to treat infectious diseases as well as lessen many of the side effects related to using synthetic antimicrobials [1,2]. The advantageous medicinal effects of plant materials mainly result from the combinations of secondary metabolites such as tannins, alkaloids and phenol compounds which are produced in the plant [3].

Traditional therapies and plant-based medications remain one of the main solutions to health problems in many developing countries [4]. Between 60 and 95% of Africans rely on traditional medication for their principal health care necessities [5,6].

A potential plant-based antimicrobial obtained from *Borassus aethiopum* is worth investigating. *B. aethiopum* (Mart) is also known as African fan palm [7]. It is a non-domesticated plant, a dioecious palm tree which grows naturally throughout the semi-arid to sub-humid regions of Africa, from Senegal to the Central African Republic [8]. It abounds naturally in the transitional and savannah zones of Ghana as well as the West African sub-regions [9]. It also occurs in wetter parts of the coastal areas and grassland, particularly east of the Volta Region [10]. In Ghana, the plant is referred to as 'Ago' by the Ewe, 'Omankube' by the Akans and 'Wiedzo' by the Ga [11].

All the parts of the plant including the roots, leaves, flowers and fruits are used in traditional medicine [12]. Various parts of the plant have been researched on extensively and have been shown to contribute significantly towards primary healthcare delivery [12-14]. A combination of powdered *B. aethiopum* male inflorescences with Shea butter is used as an antifungal treatment for cutaneous lesions in Burkina Faso. Patrons claim that *B. aethiopum* has diuretic properties and are also used in treatment of sexually transmitted diseases such as herpes and viral infections such as measles [12]. Earlier studies described the anti-inflammatory, antipyretic, and pro-apoptotic actions of extracts of this plant

Fig. 1. *Borassus aethiopum* fruit

and it has been documented to induce apoptosis of human colon cancer Ht-29 cells [13]. The anti-diabetic properties of the fruit pulp have been reported [14].

Microbial infections are treated with antimicrobial drugs which for some time now have led to an increase in microbial resistance and drug side effects. Moreover, these synthetic antimicrobial drugs are expensive. As resistance towards antibiotics becomes more common, a greater need for alternative treatments arises. The use of traditional treatments requires further scientifically sound evidence for the efficacy of the medicines in treating microbial infections [15].

This study provides a scientific basis for the ethnomedicinal uses of *B. aethiopum* in the treatment of microbial infections. The study also provides informational data on the effectiveness of the plant's antimicrobial activities against certain standard microbes hence a cheaper, safer and effective source of antimicrobial agent. Also, this information helps to establish that *B. aethiopum* extract is effective against both Gram negative or Gram positive bacteria.

2. MATERIALS AND METHODS

2.1 Study Site and Test Microorganisms

The evaluation of the antimicrobial activity of *B. aethiopum* was done at the Microbial Biochemistry laboratory of the Department of Biochemistry and Biotechnology, KNUST. *Salmonella typhi* ATCC 19430, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 33495, *Proteus mirabilis* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus saprophyticus* ATCC 15305 and *Staphylococcus aureus* ATCC 25923 were obtained from the Center for Plant Medicine Research (CPMR), Akuapem-Mampong in the Eastern Region of Ghana.

2.2 Sample Collection

Fresh fruits of *B. aethiopum* were bought from a market in Serkwa in the Brong Ahafo region of Ghana. The fruits were authenticated at the herbarium of the Department of Pharmacognosy, KNUST by comparing with a voucher specimen (KNUST/M2/2016/R003) deposited at the department's herbarium.

2.3 Preparation of Aqueous Fruit Extract

The method of extract preparation as described by Issaka et al. was by maceration [14]. The persistent calyxes of the fruits were removed with a knife. The fruits were then washed with distilled water and sliced into three with each slice having a seed. The pericarps were removed and the mesocarps were macerated to extract its fruit juice. The resulting extract was then freeze-dried or lyophilized. The resulting powder obtained after freeze-drying was diluted and used as the sample for the experiment.

2.4 Phytochemical Screening

The aqueous extract of ripe fruit of *Borassus aethiopum* plant was subjected to qualitative chemical screening for identification of the various classes of active chemical constituents such as tannins, saponins, cardiac glycosides, flavonoids, phenols, terpenoids and alkaloids. The phytochemical screening was done according to standard methods [16].

2.4.1 Test for terpenoids

Using the Salkowki's test, 2ml of the extract was added to 1ml of chloroform. A few drops of concentrated Sulphuric acid (H_2SO_4) were added carefully to form a layer. Colour formation was then observed and recorded.

2.4.2 Test for flavonoids

Alkaline reagent test was used to test for flavonoids. 5 ml of dilute ammonia was added to a portion of an aqueous filtrate of the extract. 1 ml of concentrated sulphuric acid was then added. The disappearance of a yellow precipitate on standing was observed and recorded.

2.4.3 Test for saponins

Using the froth test, 2 ml of the extract was added to 5 ml of distilled water in a test tube. The solution was then shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.4.4 Test for tannins

Using the Braymer's test, 2ml of the extract was mixed with 10% alcohol ferric chloride. Observations were done for brownish green or blue-black colouration.

2.4.5 Test for alkaloids

Using the Wagner's reagent, a fraction of the fruit extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine in 2 g of potassium iodide in 100 ml of water) and observed for the formation of reddish-brown precipitate.

2.4.6 Test for cardiac glycosides

Using the Keller Kelliani's test, 5 ml of the extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added. The test tube was slightly tilted and 1 ml concentrated Sulphuric acid was poured gently into the test tube. A brown ring at the interface was observed for the presence of deoxysugar characteristic of cardenolides.

2.4.7 Test for phenols

Using the ferric chloride test, a fraction of the extract was treated with aqueous 5% ferric chloride and observed for the formation of a deep blue or black colour.

2.5 Preparation of Extracts for Bioassay

Weights of 3 g, 4 g, and 5 g of the lyophilized aqueous extracts were reconstituted using 10ml sterile distilled water. These stock solutions were refrigerated until needed.

2.6 Preparation of Inoculum

Stock cultures of *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were sub-cultured unto fresh nutrient agar plates and incubated for 24 hrs at 37°C – to obtain pure working cultures. Three to five well-isolated colonies of each of the organisms having the same morphology were suspended in test tubes containing 10 ml sterilised Mueller-Hinton broth and incubated overnight at a temperature of 37°C for 14-16 hr. The overnight culture was then sub-cultured onto fresh sterilised Mueller-Hinton broth for between two to four hours to attain turbidity of 0.5 McFarland standards [17]. Positive control for the study was 1.5 g chloramphenicol (Denk Pharma, Germany) mixed with 1.5 g tetracycline (Ernest Chemist Limited, Ghana) dissolved in 100 ml distilled water to form 30 mg/ml of the mixture.

2.7 Antimicrobial Susceptibility Test

The agar well diffusion method was used in the investigation of the antimicrobial properties of the extracts, as described by Ugochukwu et al. [16]. Sterile Petri dishes were labelled accordingly. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterilised swab was aseptically dipped into the suspension, rotated severally and pressed firmly on the inside wall of the test tube above the fluid to remove excess inoculums from the swap. The solidified surface of the Mueller-Hinton agar plates was inoculated by streaking the sterile surface with the bacteria using the swab. This procedure was repeated twice rotating the Petri dish at an angle of 90 degrees to ensure even distribution of the inocula. Wells was created in the medium using sterile cork borer of the internal diameter of 4mm allowing at least, 30 mm between adjacent wells and between outer walls and the edge of the plate. Thirty, forty, and fifty percent weight by volume of the aqueous plant extract were poured into their respective holes with 30 mg/ml chloramphenicol mixed with tetracycline as the positive control. The experiment was done in duplicates for all the organisms. The plates were refrigerated for complete diffusion of the pipetted extracts after which the plates were incubated at a temperature of 37°C for 24 hours. Resulting zones of inhibition were measured using a meter rule. The average of the readings was taken of the zones of inhibition.

2.8 Data Analysis

Data was analyzed using GraphPad Prism 5 at a significant level of 0.05. Significant differences between the inhibition zones of the different concentrations of the fruit extract were analyzed and the results were placed in an ANOVA table.

3. RESULTS

3.1 Phytochemical Content

The phytochemical content of the aqueous extract of *B. aethiopum* is shown in Table 1.

3.2 Antibacterial Activity of *B. aethiopum* Fruit Extract

The varying degree of inhibitory activity by the aqueous extract against test bacteria is shown in Table 2.

Table 1. Phytoconstituents of aqueous ripe fruit extract of *Borassus aethiopum*

Phytochemical	Aqueous extract
Saponins	+
Tannins	+
Flavonoids	-
Alkaloids	-
Phenols	-
Cardiac glycosides	+
Terpenoids	+

KEY: (+) Indicates the presence of a particular phytochemical; (-) Indicates the absence of a particular phytochemical

4. DISCUSSION

The results from the phytochemical screening of the extracts showed the presence of saponins, terpenoids, cardiac glycosides, and tannins in the aqueous extract of *B. aethiopum* (Table 1). Phenol, flavonoid and alkaloids were absent from the aqueous extract.

Results from this study compare with findings from Kumar et al. [18] where alkaloids and flavonoids were absent while saponins and sterols were present in the *in vitro* studies of the antifungal and antibacterial properties of the male inflorescence of *B. aethiopum* Mart. The phytochemical contents were also comparable with that reported by Issaka et al. [14] in a previous study.

According to Amos-Tauta et al. [19] Saponins have been reported to have antibacterial property, possessing membrane permeabilising properties. They can cause leakage of proteins and certain enzymes from bacterial cell walls. Saponins are surface-active agents, which modify the permeability of the cell wall, thus, enabling the entry of toxic materials of vital components from the cell [20].

Tannins are high molecular weight biomolecules that have astringent properties. They complex with bacterial cell wall and engage in enzyme inhibition and substrate deprivation [21]. Tannin compounds inhibit microbial growth by causing the bacterial colonies to disintegrate, which results from their interference with the bacterial cell [22]. Their presence in the aqueous extract is thus justified by their structure and this can impact antimicrobial property to the plant extract.

Terpenoids are a large and diverse class of naturally occurring organic chemicals derived from five-carbon isoprene units assembled and modified in thousands of ways. Terpenoids can be thought of as modified terpenes. Wherein methyl groups have been moved or removed, or oxygen atoms added. This may explain their

Table 2. Zones of inhibition of varying concentrations of *B. aethiopum* extracts (mm ± SD) as function of bacterial strains

HiTest microbes	Zones of inhibition of extracts (mm ± SD)			Chloramphenicol. + tetracycline (30 mg/ml)
	0.3 g/ml	0.4 g/ml	0.5 g/ml	
Salmonella typhi	12.0 ± 2.0	15.5 ± 7.0	14.0 ± 4.0	34.5
Escherichia coli	11.5 ± 1.0	17.0 ±6.0	17.5 ± 1.0	37.0
Klebsiella pneumoniae	12.5 ± 1.0	16.0 ± 2.0	18.0 ± 0	20.0
Proteus mirabilis	12.5 ± 5.0	14.5 ± 1.0	15.0 ± 0.0	36.0
Pseudomonas aeruginosa	14.0 ±2.0	9.0 ± 0.0	15.0 ±0.0	NS
Staphylococcus aureus	15.5 ± 0.0	16.0 ± 2.0	16.5 ± 1.0	35.5
Staphylococcus saprophyticus	16.5 ± 7.0	19.5 ± 1.0	19.0 ± 8	43.0

KEY: ± Mean standard of deviation
NS: Non-susceptible to a particular microorganism

Table 3. The effect of *B. aethiopum* extract concentrations on Test microorganisms

Two-way ANOVA		
Source of Variation	**% of total variation**	**P value**
Test microbes	21.20	0.1106
Concentration	46.30	0.0003
Source of Variation	P value summary	Significant
Test microbes	Non-susceptible	No
Concentration	***	Yes

presence in the aqueous extract [23]. Some terpenoids act as phytoalexins, antibiotic compounds produced by plants in response to microbial challenge [23]. Studies were done by Sakandé et al. [12] on the antimicrobial activity of the *B. aethiopum* mart extract revealed the presence of terpenes in the extract and that the extract had antimicrobial activity.

The alkaloids content in plants is usually within a few percent and is in homogeneous over the plant tissues. Most alkaloids have lower solubility in water hence their absence in the aqueous extract [24].

According to Singleton [25] the glycoside portion dissolves in water and a greater number of glycoside in a cardiac glycoside confers polarity, thereby increasing its water solubility hence its presence in the aqueous extract.

All the bacterial strains were susceptible to the aqueous extract of *B. aethiopum*. However, the susceptibility for each organism was variable for different concentrations of the extract. This variation in level of activity among extracts could be due to the difference in solubility of the active ingredient in the aqueous solvent or the structural variability of the tested microorganisms. It could also be from the rate of diffusion of the different concentrations of the extract [26]. This observation is a common occurrence when dealing with crude extract having lots of constituents that play little or no role in the antibacterial activity of the crude extract [27]. Again these impurities may antagonize the antibacterial activity of the active constituent.

Results from this study compare with findings from Sakandé et al. [12] where extract (E2F2) had no activity against *S. aureus* whiles 100 mg/ml of E2F2 extract had a zone of inhibition of 12 ± 0.4 mm against *E. coli*. The antibacterial activity of the *B. aethiopum* fruit extract also compares with several other reports where antibacterial activity was demonstrated by other plant extracts. Studies by Grislene et al. [28]; Dorman and Deans [29]; Tonia and Johannes [30]; Ayfer and Turgay [31]; Saravanan et al. [32] demonstrates several plant extracts showing antibacterial activity against *S. aureus, E. coli, K. pneumoniae* among others with similarities in zones of inhibition (Inhibitory activity) to that of *B. aethiopum extract*. This warrants a need to consider the *B. aethiopum* fruit extract a potential drug source for treatment.

Some other factors that could affect the extracted phytochemicals include the nature of the plant material, its origin, degree of processing, moisture content, and particle size [33].

From the Table 2, all test microorganisms were susceptible to the varying concentrations of the extract and the control (chloramphenicol mixed with Tetracycline) except for *Pseudomonas aeruginosa*, which was non-susceptible to the control. This could be explained from the viewpoint that bacteria are resistant to most antibiotics especially chloramphenicol and tetracycline. The organism has lower membrane permeability and can efflux potent bactericides.

At a concentration of 30% of the aqueous extract, all the test bacterial strains were inhibited with zones of inhibition (ranging from 11.5 to 16.5 mm). The aqueous extract at a concentration of 40% also inhibited all the test bacterial strains but with bigger zones of inhibition (ranging from 9.0 to 19.5 mm) than those of the 30%. In all, the aqueous extract at 50% concentration inhibited all the test bacterial strains with bigger zones of inhibition (ranging from 14.0 to 19.0 mm) than the 30% and the 40%. It was however observed that antimicrobial activities are concentration dependent on all the test microorganisms. It can be said that increasing concentration of the extract was accompanied with an increase in zones of inhibition.

Generally, the degree of antibacterial activity of the plant extract varies from one test organism to the other, thus large zones of inhibition were produced by more susceptible isolates than the less susceptible isolates which are consistent with work done by Ryan [34].

All the test bacterial strains: *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* were susceptible to concentrations of 0.3 g/ml, 0.4 g/ml and 0.5 g/ml of the aqueous extract of *B. aethiopum.* Gram positive bacteria comprising of *Staphylococcus aureus* and *Staphylococcus saprophyticus* gave bigger zones of inhibition as compared to Gram negative bacteria comprising of *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Gram positive bacteria have no outer membrane but rather thick peptidoglycan wall and this could have accounted for their bigger zones of inhibition. In addition to their outer membrane, they produce fewer arrays of Beta-lactamases,

enzymes that degrade antibiotics or bacteriocides [33]. Gram negative bacteria have an outer membrane and a thin peptidoglycan wall. They also produce a large array of Beta-lactamases to degrade any substance that might interfere with bacteria wall synthesis (such as phytoconstituents) [34]. Hence have smaller zones of inhibition.

From Table 3, data analysis of the zones of inhibition (antimicrobial activity) using GraphPad Prism 5 revealed that the zones of inhibition associated with using different test microorganisms' accounts for 21.20% of the total variance and the effect is considered not significant. The zones of inhibition associated with using a different concentration of the extract accounts for 46.30% of the total variance. The P value is 0.0003 and the effect is considered extremely significant.

5. CONCLUSION

Ripe fruit aqueous extract of *B. aethiopum* contained tannins, saponins, terpenoids and cardiac glycosides. All the test bacterial strains were susceptible to concentrations of 0.3 g/ml, 0.4 g/ml and 0.5 g/ml of the aqueous extract of *B. aethiopum*. Generally, increasing concentrations of the extract was accompanied by increase in the zones of inhibition.

Also, Gram positive bacteria comprising of *Staphylococcus aureus* and *Staphylococcus saprophyticus* gave bigger zones of inhibition as compared to Gram negative bacteria comprising of *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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