

***Borassus aethiopum*, A Potential Medicinal Source of Antioxidants, Anti-Inflammatory and Antimicrobial Agents**

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Abstract

Background: *Borassus aethiopum* Mart (Family Arecaceae), commonly known as the African Fan Palm, is a tropical plant species found widely across Africa, and used globally for both medicinal and non-medicinal purposes. In this study, the anti-oxidant, anti-microbial and anti-inflammatory activities of an ethanolic extract of the fruit of *Borassus aethiopum* was investigated.

Methods: The Carrageenan induced foot edema model in chicks was used to investigate the anti-inflammatory activity of an ethanolic extract of the seed endocarp cotyledon. For the anti-oxidant activity determination, three assays were employed; namely, the total anti-oxidant capacity assay, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and the Folin-Ciocalteu's assay for total phenols. For the anti-microbial activity, the agar diffusion method was used. Phytochemical analysis to determine the types of secondary metabolites present was also conducted on the plant extract.

Results: The phytochemical screening showed the presence of alkaloid, tannins, saponins, steroids, carbohydrates triterpenoids and phenols. A dose dependent reduction in edema was observed when the extract was compared with Diclofenac and Dexamethasone. The extract had an EC₅₀ of 13.49 µg/ml DPPH scavenging activity, which is excellent when compared to the reference ascorbic acid of 140.00 µg/ml. Thus the extract was about 10 times more potent than the ascorbic acid.

Conclusion: The anti-microbial assay revealed activity against the gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The results obtained showed that *Borassus aethiopum* has anti-inflammatory and significant anti-oxidant properties, which are dose and concentration dependent respectively.

Keywords: *Borassus aethiopum*; Anti-inflammatory; Anti-oxidant; Anti-microbial

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Background

Inflammation is a manifestation of the body's mechanism of defending itself against injury and the entry of foreign materials and micro-organisms which is characterized by swelling, pain, heat, loss of function, and redness of the dermis [1]. Inflammation is beneficial but can be harmful when there is excessive production of reactive oxygen species (ROS) as a result of imbalance with biological antioxidants, which can lead to oxidative stress [2]. *Borassus aethiopum* Mart (Arecaceae) is a tropical plant species that grows widely across Africa (Figure 1A). Ornamentally, the leaves of the palm have served the basketry and mat industries. The trunk has been used in constructing bridges, and telegraphic poles due to its tough and termite resistant nature [3]. The roots, leaves, flowers and fruits are used for multiple purposes such as nutrition agents, treatment for sexually transmitted diseases (e.g., beign herpes), cutaneous fungal infections, and viral infections particularly measles [4]. The antipyretic activities of this plant have also been reported [5]. The flowers are used to treat impetigo, whereas the roots are used for asthma treatment [3]. The fruit contains sugars, provitamin A and vitamin C [6]. The sap is usually boiled immediately after extraction to make sugar or fermented to produce an alcoholic beverage. Also, the mature hard nuts are grounded and used in porridge [7] yet; anecdotal evidence suggests its folkloric anti-inflammation activity [4]. Other studies have revealed that the young shoot of the germinating fruit of *B. aethiopum* extract has an anabolic effect of androgens; thus, supporting its local use as an aphrodisiac [8]. Further, the methanolic seed coat of *Borassus flabellifer* has been shown to possess free radical scavenging action and its leaves have an effective anthelmintic activity against Indian adult earth worms [9,10]. Pectin has been

extracted from the ripe fruit of *B. aethiopum* [11]. Again starch isolated from the germinating nuts of *B. aethiopum* has been found to have low water binding capacity and limiting swelling and solubility values [12]. Chemically prepared activated carbon derived from *B. aethiopum* flower has been found to be used as adsorbent [13]. Deionized extract of *B. aethiopum* shells have been reported to be used as a precursor for activated carbon preparation [14]. The anti-fungal and anti-bacterial activities of a 50:50 dichloromethane-methanol extract of the male inflorescences of *B. aethiopum* in mice have been found [15]. However, these studies did not investigate the ethanolic extract of endocarp portion of *B. aethiopum* as potential medicinal source of antioxidants, anti-inflammatory and antimicrobial agents in Ghana, though the edible flesh seed of *Borassus aethiopum* is used by inhabitants of several communities in Ghana to treat diarrhea and muscle tremor in indigenous poultry species including Guinea fowls (*Numida meleagris*) (unpublished). It would therefore be of interest to delve into its scientific potential as an anti-inflammatory agent, as well as its anti-oxidant and antimicrobial properties.

Materials and Methods

Plant collection, authentication and extraction

The fruit of *Borassus aethiopum* (Figure 1B) was collected from Tsakoidzi in the Volta Region of Ghana. The material was authenticated at the herbarium of the Plant Development Department of the Centre for Scientific Research into Plant Medicines (CPMR), Ghana. The plant sample was given a voucher specimen number of DPHM/003/04/15. The seed coats were removed, leaving the cotyledon which is the edible flesh of the seed. It was then air-dried for two weeks, after which it was milled into coarse powder and kept in the lab until it was ready for use. Five hundred grams of the powdered plant material was placed in a stoppered/ tightly closed container containing 1 liter of 70% ethanol. The mixture was cold macerated at room temperature for a period of two days with frequent agitation until the soluble matter was dissolved. The solution was then filtered through Whatmann number 10 filter paper. The 70% ethanolic extract was concentrated in vacuo at 40°C, and further dried in an oven to give an extract yield of 1.01% w/w (weight by weight) per dried plant material.

Phytochemical analysis

Phytochemical analysis was carried out on the dried 70% ethanol extract of *Borassus aethiopum* according to the method of [16]. To test for the presence of saponins for instance, 10 mg of the extract was added to 20 ml of water and was shaken. The presence of a stable persistent froth indicated the presence of saponins.

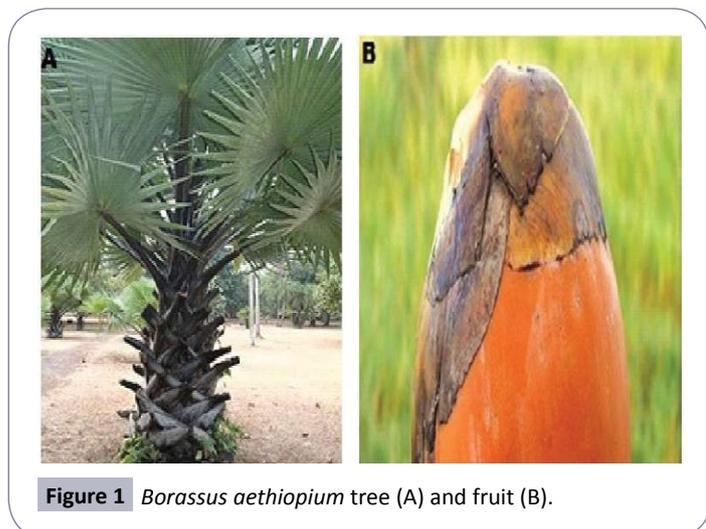


Figure 1 *Borassus aethiopum* tree (A) and fruit (B).

The standard protocol was followed to test for alkoids, tannins, glycosides, flavonoids, triterpenoids, anthracene glycosides, phytosteroids and phenols.

Anti-inflammatory studies

Experimental animals: One day post-hatch cockerels (*Gallus gallus*) were obtained from Akate Farms in Kumasi, Ghana and were housed in stainless steel cages (34 × 57 × 40 cm³) at a population density of 10 chicks per cage. Feed (Chick Mach, GAFCO) and water were available *ad libitum*. Overhead incandescent illumination was maintained on a 12 hour light-dark cycle. The room was maintained at a temperature of 29°C and relative humidity of 60 -70%. Daily maintenance of the cages was conducted during the first quarter of the light cycle. All procedures used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [17] and use of laboratory animals and was approved by the Department of Pharmacology Ethics Committee, School of Pharmacy, University of Ghana, Legon, Ghana. The chicks were used for the experiment at seven days of age. The sample size for the various groups was five.

Anti-inflammatory assay of extract: The anti-inflammatory activity was investigated using the carrageenan induced foot edema model in 7-day old chicks. This focused on the curative effect of the extract during inflammation. The initial foot volume of the chicks was measured using Vernier calliper before the injection of the 2%_{v/v} (weight by volume) carrageenan. One hour after injection, the foot volumes of the chicks were measured again. After treating them with the various extracts, the foot volumes of the chicks were measured again using Vernier calliper at an hourly interval for 5 hours, as described by [18]. The edema component of inflammation was the difference between the initial foot volume and the foot volume after the injection of the 2% carrageenan at the various time intervals. The extracts were given to the chicks orally at 30, 100, and 300 mg/kg body weight. The standard or reference drugs, dexamethasone (0.3, 1, and 3 mg/kg) and Diclofenac (10, 30 and 100 mg/kg) were also given orally. The control animals received only distilled water. The percentage inhibition of foot edema for each treatment was then calculated using the formula

$$\% \text{ inhibition of edema} = \frac{[\text{AUC control} - \text{AUC treatment}]}{[\text{AUC control}]} \times 100 \quad (1)$$

AUC-Area under curve

Anti-oxidant studies

Total antioxidant capacity assay: This assay is based on the reduction of molybdenum, Mo⁺⁶ to Mo⁺⁵, by the plant extract, followed by the formation of green phosphate-molybdate (Mo⁺⁵) complex at an acidic pH [19]. Five concentrations of vitamin C (100, 50, 25, 12.5, 6.25 µg/ml) was used to construct a calibration curve. A 1 ml of the plant extract (500, 250, 125 and 62.5 µg/ml) was added to test tubes containing 3 ml of the reagent solution (0.6M sulphuric acid, 28 mm disodium phosphate and 4 mm ammonium molybdate) and incubated

at 95°C for 90 minutes. The absorbance was determined at λ 695 nm in a spectrophotometer after the mixture was allowed to cool to room temperature. A blank solution was prepared by adding every other solution except the standard drug or extract. The antioxidant capacity was expressed as milligram (mg) of vitamin C equivalent (AAE) per gram of extract.

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity:

The free radical scavenging activity was determined as described by [20]. A 1 ml quantity of the plant extract (500, 250, 125 and 62.5 µg/ml) was added to 3 ml methanol solution of DPPH in a test tube and incubated at 25°C for 30 minutes. The absorbance of the mixture was determined at λ 517 nm in a spectrophotometer. A solution of 1 ml methanol and 3 ml of DPPH was made and incubated at 25°C for 30 minutes and used as control. Ascorbic acid (100, 50, 25, 12.5 and 6.25 µg/ml) was used as a standard free radical scavenger. Results were expressed as percentages of blank. The EC₅₀ which is the concentration required to scavenge 50% of the DPPH molecules was calculated. Each test was carried out in triplicates. The % DPPH scavenging effect (% of control) of the antioxidant was calculated as follows;

$$\% \text{ DPPH scavenging effects} = (\text{Ac} - \text{At}) / \text{Ac} \times 100 \quad (2)$$

Where, Ac=Absorbance of the control, At=Absorbance of the test drug/ extract. Concentrations responsible for 50% of the maximal effect in the DPPH assay (EC₅₀) for each drug/extract was determined.

Folin-Ciocalteu's assay for total phenols

The soluble phenol present in the extract (62.5-500 µg/ml) was quantitatively determined by the Folin-Ciocalteu's assay for total phenols [21]. Tannic acid (6.25-100 µg/ml) was used as the reference drug. A 1 ml of the test drug was added to 1 ml of Folin-Ciocalteu's reagent (diluted five times with distilled water). The content of the test tube was mixed and allowed to stand for fifteen minutes at 25°C in an oven. An amount of 1 ml of 2% sodium bicarbonate solution was added to the mixture. The reaction mixture was further incubated at 25°C for 30 minutes. The absorbance of the mixture was then determined at λ 760 nm using a UV-VIS spectrophotometer. A 1 ml of distilled water was added to one milliliter Folin-Ciocalteu's phenol reagent and the mixture processed similarly as the test drugs and used as the blank. All measurements were done in triplicates. Data obtained for the tannic acid was analyzed as linear regression of the absorbance against concentration from which the tannic acid equivalents were obtained. The data obtained were expressed graphically as column graphs of concentration against the tannic acid equivalent (Total Phenol Content) using the Graph Pad Prism for Windows version 5 (Graph Pad software, San Diego, CA, USA).

Anti-microbial assay

Agar diffusion method: The minimum inhibitory concentration (MIC) was determined using the Agar Diffusion method. A solution of the plant extract 0.1% w/v was prepared with 4% using Tween 60 and diluted to 0.001%, 0.002%, 0.004% and 0.008% w/v. A 20 ml of stabilizer nutrient agar was seeded with

100 μ l of a 24 hour broth cultured of *Escherichia coli* NCTC1351 and aseptically transferred into a sterile petri dish. The procedure was repeated using *Staphylococcus aureus* NCTC6571, clinical isolates of *Bacillus subtilis* and *Pseudomonas aeruginosa*. The agar was allowed to set. Using a 10 mm diameter cork borer, four holes were made in the agar with reasonable spacing. Each of the holes was filled with 0.1 ml of different concentration of the plant extract. The plates were pre-incubated for an hour and incubated at 37°C for 24 hours. The reference drug, gentamicin 10 μ g/ml was used as positive control.

Results

Results of phytochemical analysis

The phytochemical constituents found to be present in the extract were alkaloids, tannins, saponins, triterpenoids and phenols. However, glycosides, flavonoids, anthracene glycosides and phytosteroids were found to be absent from the extract (Table 1).

Anti-inflammatory activity

The carrageenan-induced foot edema in chicks' model was used to assess the anti-inflammatory activity of the *Borassus aethiopicum* extract. The extracts were given *per os* to the chicks at 30 mg/kg, 100 mg/kg and 300 mg/kg, 1 hour after induction of edema with 2% w/v carrageenan. Diclofenac (10-100 mg/kg, *per os*) and dexamethasone (0.1-3 mg/kg, oral administration) were used as reference drugs (Table 2). Induction of acute inflammation in control chicks resulted in a prominent increase in paw thickness, which began 1 hour after intraplantar injection of carrageenan and reached a peak of inflammation after 2 hours and slowly declined for the next 3 hours. The extract, caused significant dose-dependent inhibition of the carrageenan-induced inflammation (Figure 5) in the seven day old chicks ($P < 0.001$), the effect of which began 2 hours after carrageenan injection. Diclofenac and dexamethasone showed significant effect (Figure 6) on the time course curve ($P < 0.001$), and dose

dependently reduced the total edema induced by intraplantar injection of carrageenan (Figure 7). Diclofenac showed the highest anti-inflammatory activity, followed by the extract of *B. aethiopicum*, then dexamethasone (Table 3).

Results of anti-oxidant assays

DPPH radical scavenging activity: In this assay, both the extract and reference ascorbic acid showed a concentration-dependent scavenging effect. The DPPH scavenging activity of the extract showed a concentration dependent radical scavenging activity with EC_{50} value of 13.49 μ g/ml (Figure 2).

Total anti-oxidant capacity: In this assay, the standard drug used was vitamin C and a calibration curve was plotted as shown (Figure 3a). The anti-oxidant activity was expressed as milligram of ascorbic acid equivalent per gram of extract. The extract showed a total antioxidant activity of 329.4 mg of vitamin C equivalent per gram. The extract gave a concentration-dependent anti-oxidant activity (Figure 3b).

Total phenol content: In this assay, tannic acid was used as the standard anti-oxidant drug to develop the calibration curve (Figure 4a). The total phenol content was expressed as mg of tannic acid equivalence per gram of extract. The extract showed a concentration-dependent total phenolic content. The phenol content was 73.65 mg of tannic acid equivalent per gram of the extract (Figure 4b).

Anti-microbial results

The extract showed limited anti-bacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* (Table 4).

Discussion

This work aimed at investigating the *in vivo* anti-inflammatory and *in vitro* anti-oxidant and antimicrobial properties of the ethanolic extract of the edible portion of the seed (endosperm) of *B. aethiopicum*. The anti-inflammatory activity was targeted on the curative effect of the extract during inflammation. The carrageenan induced edema is mediated by multiple processes involving the release of inflammatory mediators. Carrageenan induced edema is a biphasic event, the first phase being attributed to the release of histamine and serotonin. This occurs during the first hour of injection of carrageenan, whereas the second phase is due to the release of lysosomes, prostaglandins and proteases, which occur after an hour [22]. A significant

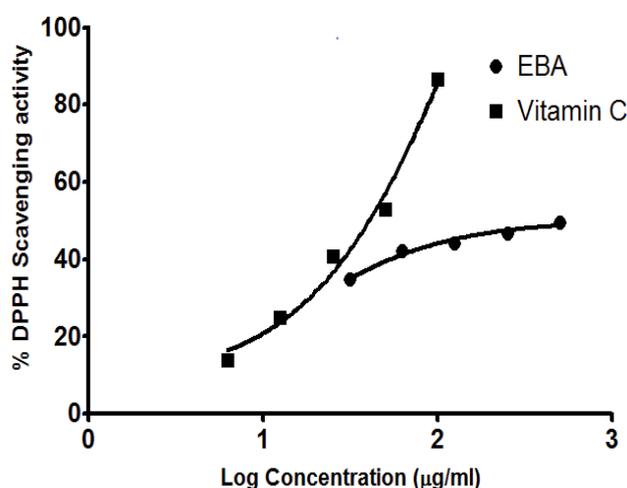


Figure 2 Percentage DPPH scavenging activity against Log concentration of ethanol extract of *Borassus aethiopicum* seed (EBA) and Vitamin C with EC_{50} values of 13.49 and 140.00 μ g/ml respectively.

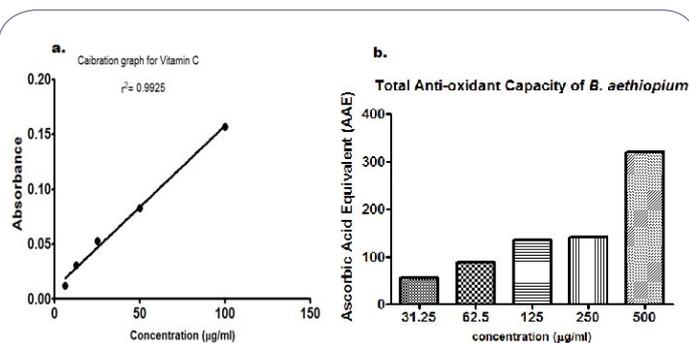


Figure 3 a. Calibration curve of Vitamin C; b. The total antioxidant capacity of the extract.

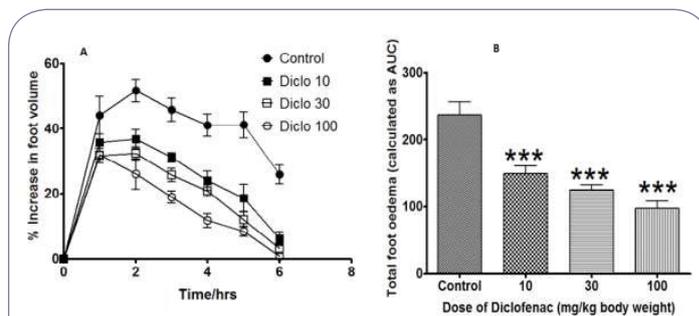
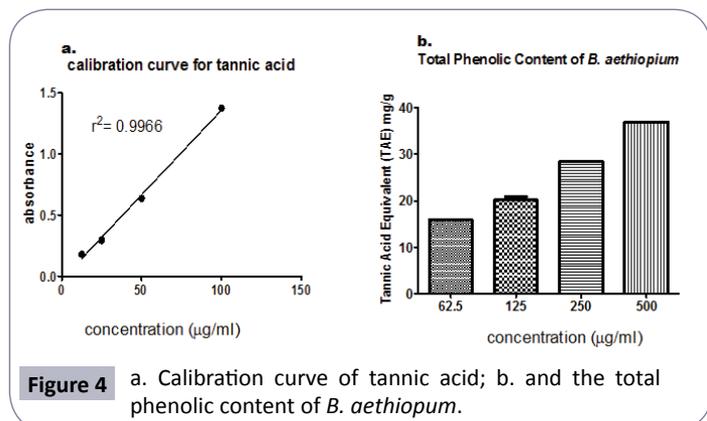


Figure 7 Effect of Dexamethasone (0.3-3 mg/kg) on time course curve. (A) The total edema response calculated as AUC's, for 6 h in carrageenan induced paw edema in chicks (B). Values are means \pm S.E.M (n=5) *** $p < 0.001$, ** $p < 0.01$. * $p < 0.05$ compared to control group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

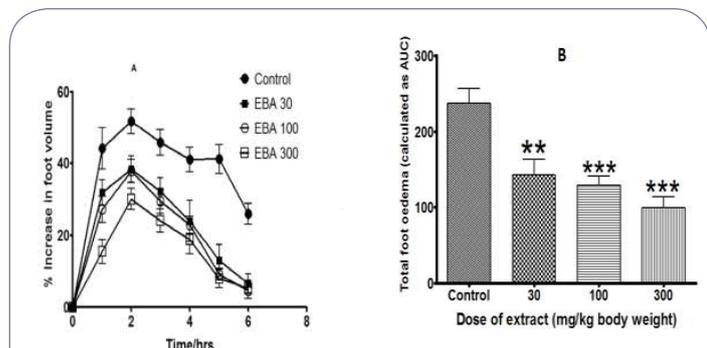


Table 1 Phytochemical constituents in the ethanol extract of *Borassus aethiopicum* (EBA).

Phytochemical Constituent	EBA
Alkaloids	+
Tannin	+
Saponins	+
Glycoside	-
Flavonoids	-
Triterpenoids	+
Anthracene Glycoside	-
Phytosteroids	-
Phenols	+

Table 2 Percentage inhibition of edema of the various drugs and their respective doses.

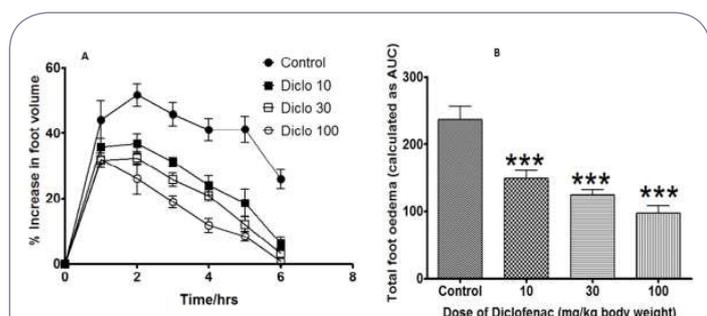
Drug	DOSE (mg/Kg)	Inhibition of edema (%)
EBA	300	58.02
Diclofenac	100	58.68
Dexamethasone	3	57.07

EBA: Ethanol extract of *B. aethiopicum*

Table 3 Anti-inflammatory activity of extract and standard drugs.

Drug	ED50 (mg/kg)
EBA	186.2 \pm 0.133
Diclofenac	30.16 \pm 0.266
Dexamethasone	3.265 \pm 0.111

Figure 5 Effect of EBA (30-300 mg/kg) on time course of edema. (A). The total edema response, calculated as AUC's, for 6 hours, in carrageenan induced paw edema in chicks (B). Values are means \pm S.E.M (n=5), *** $p < 0.001$, ** $p < 0.01$. * $p < 0.05$ compared to control group (One-way ANOVA followed by Newman-Keul's *post hoc* test).



aethiopicum has anti-inflammatory properties. This may explain the ethno-veterinary use of *B. aethiopicum* in treatment of edema and tremor in muscles in Guinea fowls during preliminary stages of this study (unpublished). Thus, there is the need to further investigate the bioactive constituents that may be responsible for the effect on inflammation. Inflammation is implicated in a number of disease conditions such as arthritis, inflammatory bowel disease, cardiovascular disease, metabolic syndrome, diabetes, obesity, autoimmune disease, dementia, osteoporosis, cancer and asthma [24]. The plant extract showed the presence of triterpenoids, saponins, tannins, phenols and alkaloids. Other researchers have shown that different phytochemicals possess a wide range of activities, which may help in the protection

($p < 0.01$) anti-inflammatory effect was produced by the maximum concentration of the extract used (300 mg/kg). This significant inhibition was seen in the second hour of inflammation, indicating that the extract produced its anti-inflammatory effect probably by inhibiting the release prostaglandins, stabilizing the lysosomal membrane or by inhibiting the release of lysosomal enzyme [23]. Results showed that the extract produced a dose dependent anti-inflammatory effect. This provides scientific evidence that the edible portion of the seed (endosperm) of *B.*

Table 4 Zones of inhibition of the plant extract and standard drug.

Concentration of extract (% ^w / _v)	Average Zones of Inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
0.008	12.0 ± 0.03	18.5 ± 0.02	14.9 ± 0.03	14.4 ± 0.02
0.004	11.3 ± 0.32	14.5 ± 0.01	14.0 ± 0.03	12.0 ± 0.04
0.002	11.0 ± 0.00	13.5 ± 0.01	12.9 ± 0.03	11.0 ± 0.03
0.001	10.0 ± 0.00	10.9 ± 0.14	12.0 ± 0.01	10.1 ± 0.06
Gentamycin 10 µg/ml	16.0 ± 0.00	15.8 ± 0.07	16.9 ± 0.07	16.7 ± 0.01

against inflammatory diseases [25,26]. Glycosides, saponins, flavonoids, tannins, alkaloids and triterpenoids have been reported to possess anti-inflammatory activities and therefore could be responsible for the anti-inflammatory activity of the extract. Chronic inflammations can lead to oxidative stress [27]. Therefore the anti-oxidant activity of the extract was investigated using the DPPH radical scavenging activity and the total anti-oxidant capacity assays. In the DPPH scavenging activity the extract showed a concentration dependent radical scavenging activity with EC₅₀ value of 13.49 µg/ml, whereas that of ascorbic acid, was 140.0 µg/ml. Thus the extract was about 10 times more potent than that of ascorbic acid. In an inflammatory process, there is excessive production of ROS as a result of imbalance of biological antioxidants which can lead to oxidative stress. Also reactive oxygen metabolites from phagocytic leukocytes may invade body tissues causing injury to them [2]. As a matter of fact plants that contain compounds that have the ability to scavenge these reactive oxygen species play significant role in treating and managing inflammation [23].

The extract showed a total antioxidant activity of 329.4 mg of Vitamin C equivalent per gram of extract whereas the total phenol content was 73.65 mg of tannic acid equivalent per gram. Phenolic compounds have the ability to scavenge free radicals, which makes them good anti-oxidants [27]. From the correlation between the total phenolic content and the total anti-oxidant capacity, a coefficient of 0.8270 was obtained. This value indicates that 82.70% of the anti-oxidant activity of the extract may be as a result of its phenolic content. The remaining 17.30% of its anti-oxidant activity can be attributed to other secondary metabolites present in the extract. It can be deduced from the results that, the anti-oxidant property of the extract might have helped in delaying and curing the inflammatory process.

The plant extract exhibited broad spectrum antibacterial activity against *B. subtilis* and *S. aureus*. However, at 0.008%

w/v concentration of the extract, high zones of inhibition were observed against *E. coli* and *P. aeruginosa*. Gram-negative bacteria are generally more resistant compared to the Gram-positive ones [28]. Alkaloids, saponins, phenols, tannins and triterpenoids found in the phytochemical screening may have contributed to the antimicrobial activity observed. The importance of alkaloids, saponins and tannins in various as antibiotics used in treating common pathogenic strains has been reported [29,30]. Alkaloids have been found to intercalate into cell wall and DNA of parasites, triterpenoids also cause membrane disruption whereas tannins bind to adhesions, inhibit enzyme inhibition, cause substrate deprivation, complex with cell wall, cause membrane disruption, and metal ion complexation [31]. In the process of wound formation there is a release of inflammatory cytokines and reactive oxygen species. Wounds as a matter of fact predispose one to opportunistic infections and as such antimicrobials also play a significant role in wound management. Since extract of *B. aethiopicum* showed activity against *S. aureus* and *B. subtilis* (which are commonly found in infected sores and wounds), it may be useful as a wound healing agent in human and ethno-veterinary medicine.

Conclusion

The 70% ethanolic extract of the seed (endosperm) of *B. aethiopicum* exhibited both dose dependent anti-inflammatory and concentration dependent anti-oxidant activities. It also possessed antimicrobial effect against both Gram-positive and Gram-negative bacteria. The prominent anti-oxidant activity of the extract may provide cytoprotection of cells against the ROS produced during inflammation. Its anti-microbial activity is also likely to reduce the extent of inflammation during infection. These results seem to justify the folkloric use of the plant material for the treatment of infections and possibly some inflammatory conditions in both humans and animals.

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