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Anti-inflammatory and Anti-infective Properties of Ethanol Leaf and Root Extracts of *Baphia nitida*

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Authors' contributions

This work was carried out in collaboration between all authors. Author CA conceived and designed the study, wrote the protocol and the first draft of the manuscript. Authors SO, EOB, LAA and YDB performed the phytochemical investigations, anti-inflammatory and anti-infective studies, statistical analysis and managed the analyses of the data. All authors read and approved the final manuscript.

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ABSTRACT

Baphia nitida Lodd. (Family Leguminosae-Papilionoideae) is a tropical plant used in African folkloric medicine for the treatment of infections and inflammatory conditions. This study therefore seeks to investigate the biological activities including antimicrobial, antioxidant and anti-inflammatory properties of 70% v/v ethanol leaf and root extracts of *B. nitida.*

The phytochemical constituents of the ethanol root and leaf extracts of *B. nitida* was determined by thin layer chromatography (TLC) analysis and *in vitro* phytochemical tests, while their anti-microbial activities were determined using the agar diffusion and micro-dilution methods. The antioxidant and anti-inflammatory activities of this extract was also investigated by the DPPH radical scavenging methods and *in vivo* carrageenan-induced paw oedema model of inflammation, respectively.

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The antioxidant properties of the extracts as determined by the DPPH free radical scavenging assay gave IC_{50} values for reference α-tocopherol, *B. nitida* root and leaf extracts as 1.216, 4.656 and 279.4 μg/mL, respectively. The ethanol root extract was further investigated for its anti-inflammatory property. The root extract demonstrated potent anti-inflammatory activity at low concentrations of between 50 - 200 mg/kg body weight as compared to the highest concentration of 400 mg/kg. The extract had a significant decrease in foot weight when compared to the negative control. At 800 mg/kg, significant anti-inflammatory activity was observed with a better reduction in the paw oedema than the positive control (aspirin). The extract also exhibited broad spectrum antimicrobial activities against *C. albicans*, *S. aureus, B. subtilis*, *P. aeruginosa* and *E. coli*.
Combination of antimicrobial, antioxidant and anti-inflammatory properties of the plant may be responsible for its traditional uses in the treatment of infections, wounds and inflammatory conditions.

Keywords: Infections; wounds; antimicrobial; antioxidant; anti-inflammatory.

1. INTRODUCTION

Inflammation is a normal part of the body's process aimed at removing foreign materials such as contaminating micro-organisms and threats from toxins, environmental pollutants, injury, stress and other harmful influences. This natural defense mode works to shield the body's systems and initiate the healing process. Inflammation is beneficial; however, when one's body is in a chronic state of inflammation, it can have serious effects on your cellular health. Chronic inflammation has been linked to degenerative diseases including cancer, heart disease, diabetes, Alzheimer's and many others. When inflammation occurs, inflammatory cytokines and reactive oxygen species are released into the blood or tissues as part of the healing response. Excessive inflammatory cytokines are destructive to our normal cells [1-3] and in chronic inflammation they result in irritation and wearing down of cartilages, tissues, and lead to further inflammatory triggers [4]. Excessive production of oxygen species will also lead to oxygen radicals destroying tissues, extracellular matrix and growth factors [5].

In the absence of effective decontamination, inflammation may be prolonged. Both microbes and their endotoxins can lead to a persistent elevation of pro-inflammatory cytokines such as IL-1 and TNF-α thus prolonging the inflammatory phase. If inflammation continues, a wound may enter a chronic state leading to increased levels of matrix metalloproteases that can degrade the extracellular matrix. Correlating with this is a decrease in the level of protease inhibitors. This shift in protease balance can cause growth factors in chronic wounds to be rapidly degraded [6,7]. The absence of growth factors lead to a decline in tissue regeneration. Multipurpose plant-based remedies such as *Baphia nitida* provide an alternative means for the management of inflammation as well as infection simultaneously.

Baphia nitida Lodd. (Leguminosae-Papilionoideae) is one of the species of *Baphia,* known locally as 'onwono' in the Asante Twi language in Ghana. It is a shrub which grows to a height of about 9 m [8]. Geographically, it is found in the wetter parts of the coastal regions, the rain and secondary forests and on abandoned farmland from sea-level up to 600 m altitude. Various parts of *B. nitida* has been used by indigenes of many West African countries for a wide range of ethno-medicinal purposes [9,10] and often also used for ornamental purposes [8]. *B. nitida* is used to treat constipation [9], ringworm, sprains and swollen joints, parasitic skin diseases, wounds, ulcers, boils, venereal diseases, and gastrointestinal problems [11,12]. In Ghana, Côte d'Ivoire and Nigeria, the leaves and bark are considered haemostatic and anti-inflammatory and therefore used to treat healing wounds, inflamed and infected umbilical cords [13]. *B. nitida* has also been shown to have analgesic and hepatoprotective activities which are highly correlated to its antioxidant activities [14]. Such plants with anti-inflammatory potentials are largely preferred because of their widespread availability, less or no side effects, and their effectiveness as crude preparations [15] in the treatment of inflammatory conditions. In Nigeria, powdered heartwood is applied as an ointment to stiff and swollen joints and to treat sprains and rheumatic complaints [13]. Specific compounds isolated from this plant include isoflavonoid-flavonoid dimers santalins A and B and santarubins A, B and C, baphic acid, baphiin, deoxysantarubin, homopterocarpin, maackiain, pterocarpin and santal [13]. The aim of the study is to investigate some of the biological activities including antimicrobial,

antioxidant and anti-inflammatory properties of ethanol leaf and root extracts of *B. nitida.*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh leaves and roots of *B. nitida* were collected between August 2013 and January 2014 from Kotei in the Ashanti Region of Ghana and authenticated. A voucher specimen of *B. nitida* with number AA 56 has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

2.2 Extraction of Plant Materials

Fresh roots and leaves of *B. nitida* were air dried at room temperature (28 to 30C) for two weeks and pulverized using the lab mill machine (Christy and Norris, Chelmsford, England). Two hundred and thirty grams (230 g) each of pulverized plant material was extracted with 300 mL of 70% ethanol by Ultra-turrax extraction for 5 min at 24 $\times 10^6$ rpm. The extract was filtered from the residue, concentrated in a rotary evaporator (VWR, IKA, Germany) at 40℃ and lyophilized.

2.3 Preliminary Phytochemical Analysis

The 70% v/v ethanol root and leaf extracts of *B. nitida* were analyzed by TLC after dissolving in methanol and developing on silica gel 60 F_{254} plates (10×20 cm) with petroleum ether and ethyl acetate (9:1) as the mobile phase. Documentation was done before and after derivatisation with anisaldehyde-sulphuric acid as a universal detection reagent. Visualization of the spots was at both the short and long wavelengths of λ 254 and 366 nm, respectively. Further *in vitro* phytochemical screening of the plant extracts were done to determine the main plant secondary metabolites present in the plant materials, namely alkaloids, saponins, tannins, anthracene glycosides, flavonoids, coumarins, sterols, cyanogenetic glycosides, cardiac glycosides, according to the method described by Evans [16] and Harborne et al. [17].

2.4 Antimicrobial Activities of the Extracts

2.4.1 Test microorganisms

Pseudomonas aeruginosa ATCC 4853*, Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923*, Bacillus subtilis* NTCC 10073 and clinical isolates of *Candida albicans* were obtained from Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

2.4.2 Agar well diffusion method

Determination of antimicrobial activity of the 70% v/v ethanol leaf and root extracts of *B. nitida* was performed according to the agar well diffusion method described by Agyare et al. [18]. *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* were seeded into 20 mL molten nutrient agar (Oxoid Limited, UK) with 1 x 10^5 cfu/mL each test orgainism. One hundred (100) microlitres of sterile test extracts dissolved in sterile distilled water were then introduced into wells of 10 mm in diameter using cork borer No. 5 and pre-incubated for 1 h to allow for adequate diffusion. Extracts were tested at concentrations of 200, 150, 100 and 50 mg/mL with 5 mg/mL of ciprofloxacin (Sigma-Aldrich, Steinheim, Germany) as the reference antibacterial agent. Incubation was done at 37℃ for 24 h and the zones of growth inhibition recorded. The antifungal activity was determined using similar procedure but with *C. albicans* as the test organism and 20 mL Sabouraud dextrose agar (Oxoid Limited, UK) used as medium for growth of the test organisms and incubated 3 days at 28 to 30℃. All test samples were prepared by dissolving the extracts in sterile distilled water. Clotrimazole (Sigma-Aldrich, Steinheim, Germany) 5 mg/mL was used as the reference antifungal agent. Sterile distilled water served as the negative control.

2.4.3 Micro-dilution method

The minimum inhibitory concentrations of extracts against the test organisms were determined according to Eloff [19] and Agyare et al. [18] with slight modifications. Test solutions of the plant extracts were introduced into 96 well plates and serially diluted using the required volume of sterile distilled water. One hundred microliters of double strength nutrient broth (Oxoid Limited, UK) and 20 µL of 1 x 10^6 cfu/mL of a 24 h old culture of *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* were added to each well to make the volume up to 200 μL to obtain concentrations of 25, 50, 100 and 200 mg/mL. The plates were pre-incubated for 30 min and further incubated at 37℃ for 24 h. To detect microbial growth, 10 µL MTT reagent (3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma-Aldrich, Steinheim, Germany) of concentration 1.25 mg/mL was added to each of the wells and incubated at 37℃ for 10 min [19]. Wells with bacterial growth showed the formation of blue formazan due to the reduction of yellow MTT by mitochondrial dehydrogenase [20]. The least concentrations that inhibited microbial growth were recorded as the MIC [21]. This procedure was then repeated for ciprofloxacin (Sigma-Aldrich, Michigan, USA) and clotrimazole (Sigma-Aldrich, Michigan, USA) as the reference drugs.

2.5 Antioxidant Activity of the Extracts

Determination of the free radical scavenging activity was carried out according to the method described by Chizzola et al. [22] and Agyare et al. [23]. 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma-Aldrich, Taufkirchen, Germany) solution of 0.002% in methanol was prepared and 3 mL of this solution was added to 1 mL of the test extracts dissolved in 70% v/v methanol at concentrations of 100, 30, 10, 3, 1, 0.3, 0.1 μg/mL (A_T). Different concentrations of α-tocopherol (Sigma-Aldrich, Taufkirchen, Germany) were also prepared in methanol and used as reference antioxidant. The mixture was mixed and incubated for 30 min under light protection, after which absorbance was taken at λ 517 nm with 70% v/v methanol as the blank using a spectrophotometer (Cecil CE 7200, Cecil instrument Limited, Milton Technical Centre, England). Absorbance of the DPPH solution was measured to serve as the control (A_C). Inhibitions of free radical scavenging activities were calculated using the formula:

$$\text{Inhibition (\%)} = [(A_C - A_T)/ A_C] \times 100\%$$

where A_C is the absorbance of the control, A_T is the absorbance of the test sample at λ 517 nm and Inhibitory Concentration, IC_{50} is the concentration [μg/mL] reducing the absorbance by 50%.

2.6 *In vivo* Anti-inflammatory Assay

2.6.1 Experimental animals

One day old Cockerels (*Gallus gallus;* strain Shaver 579) weighing from 40 to 60 g, were obtained from, Akropong Farms, Kumasi, Ghana and housed in stainless steel cages (34×57×40 cm^3) containing saw dust as bedding. The cockerels were randomly grouped into groups of five chicks per cage and fed *ad libitum* with animal feed (Chick Mash, Gafco, Tema, Ghana) and water. Room temperature was maintained at 29℃ with overhead incandescent illumination on a 12 hour light-dark cycle. Daily maintenance of the cages was conducted during the first quarter of the light cycle. Tests were conducted when the chicks were 7 days old. Handlings of the animals in this study were in accordance with the guidelines published by the National Institute of Health for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The research protocol was approved Faculty of Pharmacy Ethical Review Committee, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

2.6.2 Carrageenan-induced paw oedema model of inflammation

The carrageenan-induced oedema model in the footpad of chicks, as described by Roach and Sufka [24] and modified by Woode et al. [25] was used to evaluate the anti-inflammatory properties of the ethanol 70% extracts of the leaves and roots of *B. nitida*. The initial foot volumes of the chicks were measured before the extracts and carrageenan sodium salt were administered. The leaves extract (200, 400 and 800 mg/kg) as well as the standard drug (aspirin at 5 mg/kg) was orally administered. After 30 min, 10 µL of a 1% suspension of carrageenan (Sigma Chemicals, St. Louis, MO, USA) prepared in normal saline was injected sub-plantar into the right footpads of the chicks. The foot volume was measured at hourly intervals for 6 h after injection of the carrageenan. The control animals received only normal saline (0.9% w/v). The procedure was repeated for the roots extract using doses of 50, 100 and 200 mg/kg. These concentrations were used because they were determined to be the active concentrations through previous preliminary studies.

2.7 Statistical Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Data are presented as mean ± SEM (N=5) and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P< 0.05$, $^{**}P< 0.01$ and $^{***}P< 0.001$: The IC_{50} was generated using Graph Pad prism version 5 for windows.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

Phytochemical analysis of the 70% ethanol extract of *B. nitida* leaves and roots were performed by TLC analysis and phytochemical tests. After detection with anisaldehyde-sulphuric acid reagent, the TLC plates showed the presence of phenols, terpenes, sugars, and steroids by forming violet, blue, grey or green bands under day light (Fig. 1, for ethanol 70% extract of *B. nitida* root) while typical blue and red fluorescent zones formed at λ 357 nm are indicated for saponins and fatty acids [26]. The TLC analysis of the leaf extract showed similar fingerprint profiles. Phytochemical analysis was further investigated by *in vitro* phytochemical screening and these showed the presence of tannins, glycosides, saponins, flavonoids and sterols for both the root and leaf extracts (Table 1).

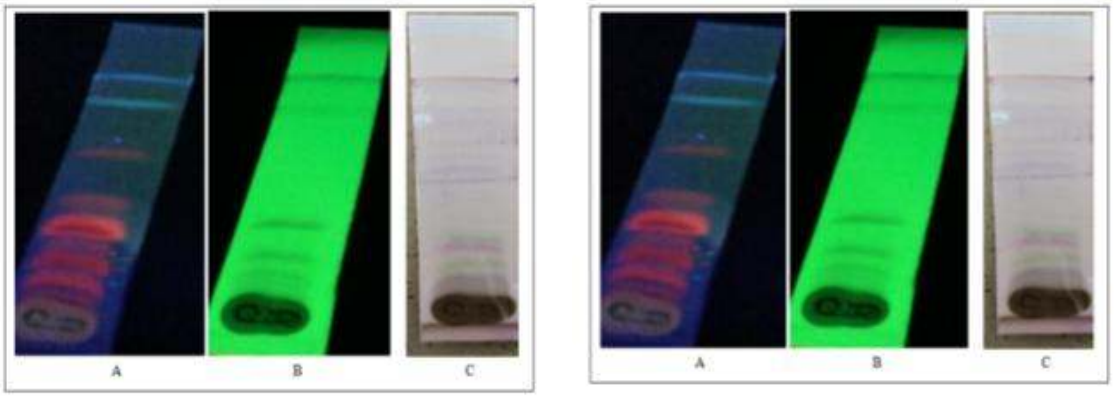
3.2 Antimicrobial Activity

The antimicrobial activity of the ethanol leaf and root extracts of *B. nitida* was determined against a panel of microorganisms, two Gram-positive bacteria (*S. aureus* and *B. subtilis*), two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and one fungus (*C. albicans*), using the cup plate agar diffusion method. The ethanol extracts of the plant were active against all the tested organisms at concentrations of 50, 75, 100 and 200 mg/mL, giving zones of inhibition as recorded in Table 2. The minimum inhibitory concentrations (MIC) of the ethanol leaves extract of *B. nitida* against *C. albicans*, *S. aureus, B. subtilis*, *P. aeruginosa* and *E. coli* were equally 50 mg/mL, that for the root extract were 50 mg/mL, 50 mg/mL, 50 mg/mL, 100 mg/mL and 200 mg/mL, respectively (Table 2). The MICs obtained, indicates that the ethanol leaf and root extracts have broad spectrum activity against bacteria and fungi at high concentrations with the ethanol leaf extract showing better antimicrobial activity against the test bacteria and fungus.

Table 1. Phytochemical constituents of the 70% ethanol extracts of the leaves and roots of *B. nitida*

Phytochemical constituents	Leaves extract	Roots extract
Tannins	+	+
Glycosides	+	+
Saponins	+	+
Alkaloids	-	-
Flavonoids	+	+
Coumarins	-	-
Sterols	+	+
Anthracene glycosides	-	-
Cardiac glycosides	-	-

Key: + = present of metabolites; - = absent of metabolites



I **II**

Fig. 1. TLC chromatograms of ethanol (70%) root (I) and leaf (II) extracts of *B. nitidia* at λ 357 nm (A) , λ 254 nm (B) and at daylight after derivatisation with anisaldehyde sulphuric acid (C)

Table 2. Mean zones of growth inhibition of ethanol root and leaf extracts of *B. nitida* and reference compounds

Concentration [mg/mL]	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Candida albicans*
Mean zones of growth inhibition (mm) of *B. nitida* root *extract*					
200	17.5±0.7	18.5±0.7	14.0±0.0	18.0±1.4	19.0±0.0
100	16.5±0.7	17.5±0.7	13.0±0.0	17.0±1.4	15.0±0.0
75	14.0 ±0.0	16.0±1.4	0.0	15.5±2.1	15.0±0.0
50	12.8±0.3	11.5±0.7	0.0	12.5±0.7	13.5±0.7
Mean zones of growth inhibition (mm) of *B. nitida* leaf *extract*					
200	27.5 ±3.5	35.0±0.0	22.5±3.5	22.5±3.5	30.5±0.7
100	26.0 ±1.4	29.0±1.4	20.0 ±0.0	21.5 ±0.7	32.0 ±0. 0
75	23.5 ±0.7	29.0±0.0	19.5 ±0.7	25.0±0.0	29.0±0.0
50	20.0 ±0.0	27.5±0.7	18.0 ±0.0	20.0 ±0.7	25.0 ±0.0
Ciprofloxacin(mm)					
1	38.5±0.5	38.0±0.0	41.0±1.0	39.5±2.5	nd
0.5	36.5±0.5	35.5±0.5	34.5±0.0	39.5±0.5	nd
0.25	31.5±0.5	32.5±0.5	32.0±0.0	35.0±1.0	nd
0.125	33.5 ±0.5	29.0±1.0	29.5±0.5	32.0±2.0	nd
Clotrimazole(mm)					
5	nd	nd	nd	nd	40.0±0.5

nd: not determined

Table 3. Minimum inhibitory concentration (MIC) of the ethanol root and leaf extracts of *B. nitida* and reference compounds

Test organism	ELBN (mg/mL)	ERBN (mg/mL)	Ciprofloxacin (mg/mL)	Clotrimazole (mg/mL)
S. aureus	50.0	50.0	0.2	_
E. coli	50.0	200.0	0.4	_
B. subtilis	50.0	50.0	0.1	_
P. aeruginosa	50.0	100.0	0.03	_
C. albicans	50.0	50.0	-	5.0

ELBN=Ethanol leaf extract of B. nitida; ERBN = Ethanol root extract of B. nitida

3.3 Antioxidant Activity of the Extracts

The inhibitory concentration (IC_{50} values) obtained in the *in vitro* DPPH radical scavenging activity assay for α-tocopherol and the ethanol leaf and root extracts were 1.216, 279.4 and 4.656 μg/mL respectively. The free radical scavenging graph (Figs. 2 and 3) showed that the reference antioxidant was more potent than *B. nitida* extracts, with the root extract having a more antioxidant activity than the leaf extract.

3.4 *In vivo* Anti-inflammatory Assay

Induction of acute inflammation in the chicks resulted in a prominent increase in paw thickness, which began 1 h after intraplantar injection of the carrageenan and reached peak inflammation after 2 h (Figs. 4 and 5) after which inflammation slowly declined with time, for the 6 h duration of the experiment. Oral administration of the ethanol root extract of *B. nitida* (50, 100 and 200 mg/kg) resulted in a reduction in foot volume from the second hour when compared to the control group (Fig. 4), thus presumably inhibited the synthesis and release of inflammatory mediators that would have been released. The reduction in the increase in foot volume was not dose dependent. At 100 mg/kg, the reduction in foot volume was less significant ($p = 0.04$) as compared to the reduction in foot volume after administration of 50 mg/kg ($p = 0.01$). The reduction in foot volume was more significant with the 200 mg/kg ($p = 0.003$) thus showing a non-linear relationship between the dose of the ethanol root extract and percentage increase in oedema (Fig. 4). The administration of the ethanol leaf extract of *B. nitida* at higher concentrations (200, 400 and 800 mg/kg) reduced footpad oedema formation from

the second hour (Fig. 5), presumably also inhibiting the synthesis and release of inflammatory mediators at those concentration. The reduction in foot volume was dose independent. At 400 mg/kg, the reduction in foot volume was not significant (p = 0.98) as compared to that of the 200 mg/kg (p = 0.015) and 800 mg/kg (p = 0.003) which were significant, thus showing a non- linear relationship between the dose of the ethanol leaf extract and percentage decrease in foot volume (Fig. 5). The root extract was more potent than the leaf extract because at relatively lower doses (50 to 200 mg/kg) similar anti-inflammatory effects was seen as compared to the leaf extract (200 to 800 mg/kg). There was no significant difference in anti-inflammatory or anti-oedematogenic effect between the leaf and root extracts of *B. nitida* and *aspirin* on foot pad oedema induced by carrageenan.

4. DISCUSSION

The phytochemical screening of the dried leaves and roots revealed presence of tannins, glycosides, saponins, flavonoids and sterols of which any could be responsible for the physiological action on the human body [21]. Antioxidants which scavenge reactive oxygen radicals have been found to complement the anti-inflammatory process, promote tissue repair and wound healing [27]. The antioxidant activities of extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes [28]. The constituents of the extracts, such as tannins and flavonoids, may play a major role in the anti-inflammatory process by preventing and protecting against oxidative damage from free radicals [29]. Tannins are generally known to have natural anti-inflammatory and antioxidant

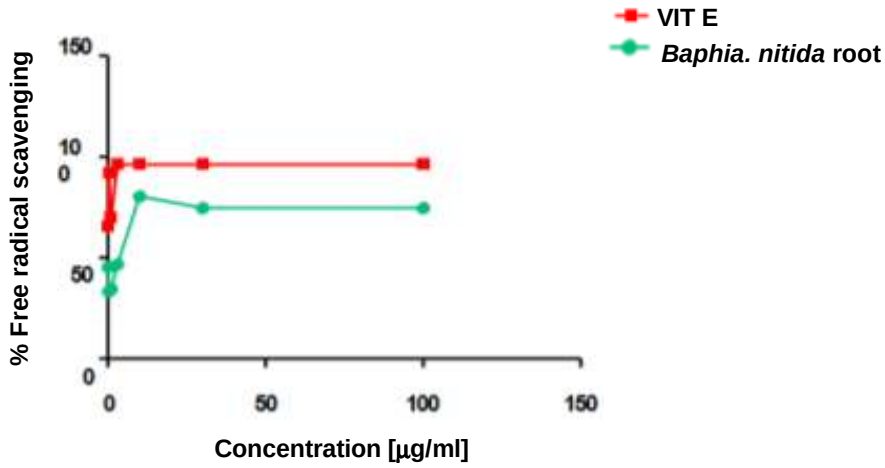


Fig. 2. Free radical scavenging activity of α-tocopherol and the ethanol extract of *B. nitida* root

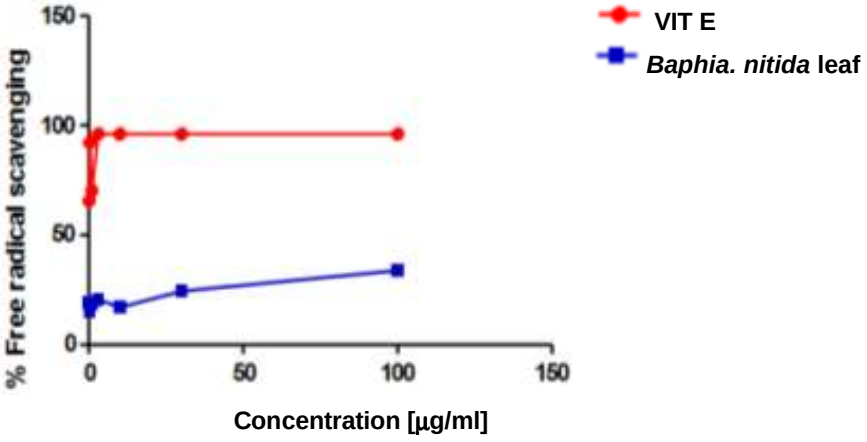


Fig. 3. Free radical scavenging activity of α-tocopherol and the ethanol extract of *B. nitida* leaf

actions [30]. The ethanol extracts of the plant showed broad spectrum antimicrobial activity against all tested microorganisms. The MICs of the ethanol leaf of *B. nitida* against *C. albicans*, *S. aureus, B. subtilis*, *P. aeruginosa* and *E. coli* were 50 mg/mL respectively while that for *B. nitida* root were 50, 50, 50, 100 and 200 mg/mL, respectively indicating that the leaf extract has better activity against especially the Gram-negative bacteria. The antimicrobial action of the extracts may also be attributed to the astringent or non-specific actions of the phenolic constituents including tannins and other polyphenols present in the extracts [31]. This may therefore justify the use of these extracts for the treatment of various bacterial and fungal infections such as ringworm and venereal diseases.

DPPH radical scavenging assay suggests that both the root and leaf extracts have the ability to mob up oxygen radicals which could complement the anti-inflammatory process and promote tissue repair [32] hence *B. nitida* root extract which had a lower IC_{50} showed higher antioxidant activity as compared to the leaf extract. Carrageenan-induced acute footpad edema has been generally used to screen new anti-inflammatory drugs and it is established to be biphasic [33]. The early phase (1 to 2 h) of the carrageenan model is chiefly mediated by serotonin, histamine, and increased synthesis of prostaglandins in the damaged tissues. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [1-3]. The ethanol leaf and root extracts of *B. nitida* inhibited the inflammation induced with carrageenan in both phases. Although the specific means of action of the extracts in inflammation inhibition is unknown, it is possible that the extracts inhibit the synthesis and release of inflammatory mediators involved in carrageenan-induced edema which include cytoplasmic 30 enzymes, serotonin, bradykinin, prostaglandins and other cyclooxygenase products [33]. The ethanol root extract was more potent than the ethanol leaf extract being active at lower doses (50 - 200 mg/kg) than the ethanol leaf extract (200 - 800 mg/kg).There was no significant difference in anti-inflammatory activity between the leaf and root extracts of *B. nitida* and *aspirin* which is the positive control.

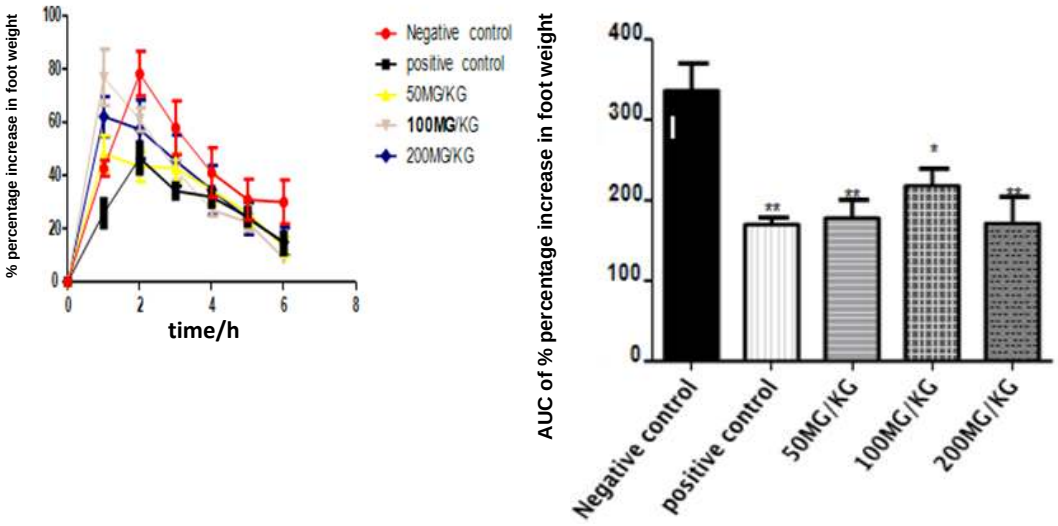


Fig. 4. Effect of the ethanol root extract of *B. nitida* (50, 100, 200 mg/kg body weight) and Aspirin (5 mg/kg) on the (a) time course curve and (b) AUC of percentage increase in foot weight

*Negative control = normal saline. Test drugs: Significant from normal control, * p < 0.05; ** p< 0.001. Mean ± SEM = Mean ± standard error of mean (SEM), n= 6*

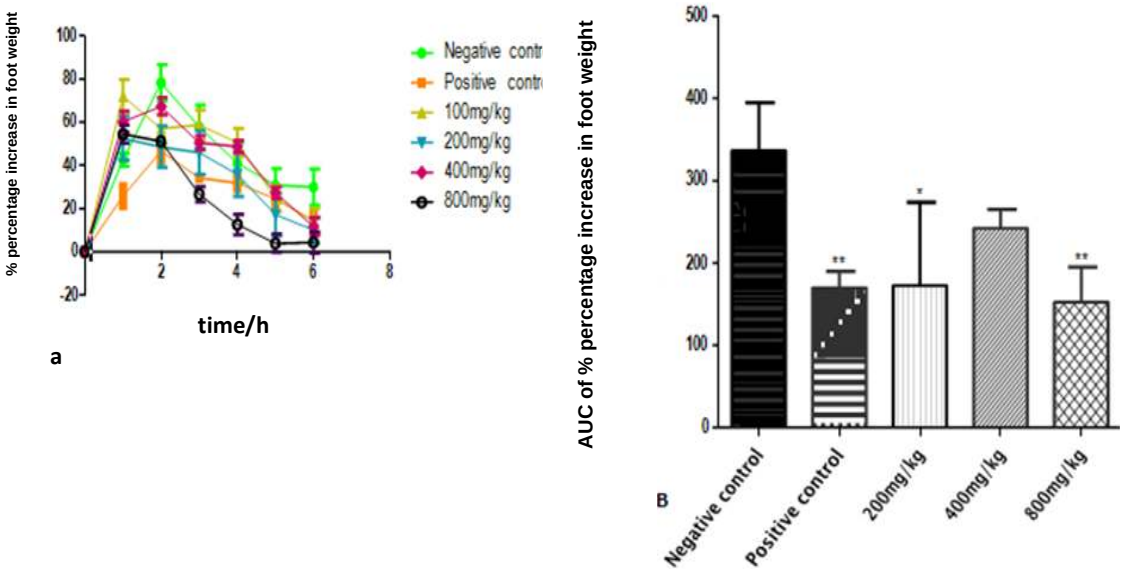


Fig. 5. Effect of the ethanol leaf extract of *B. nitida* (100, 200, 400 and 800 mg/kg body weight) and aspirin (5 mg/kg) on the (a) time course curve and (b) AUC of percentage increase in foot weight

*Negative control = normal saline. Test drugs: Significant from normal control, * p < 0.05; ** p< 0.001. Mean ± SEM = Mean ± standard error of mean (SEM), n= 6*

Flavonoids have been identified to inhibit the action of prostaglandins which are involved in the last phase of acute inflammation [34]. The presence of tannins also presupposed that the extract will have anti-inflammatory activity. Literature data state that the anti-inflammatory effect of tannins occurs through inhibition of various signaling pathways such as mitogen-activated protein kinase and NF-κB signaling pathways, which are two factors that play a major role in controlling inflammation [35]. The presence of these phytoconstituents suggested that the observed anti-inflammatory activity may be due to the presence and actions of one or a combination of the flavonoids, tannins and glycosides present in the plant extracts. The above findings may justify the folkloric use of *B. nitida* for the treatment of inflammatory conditions, wounds and infections. However, further phytochemical studies are needed to isolate the active compound (s) responsible for these pharmacological activities.

5. CONCLUSION

Ethanol leaf and root extracts of *B. nitida* have antimicrobial, antioxidant and exhibited considerable anti-inflammatory activities. The ethanol root extract at lower doses exhibited anti-inflammatory properties. Ethanol leaf extract of *B. nitida* showed better antimicrobial activity than the root extract. Both the leaf and root extracts of *B. nitida* exhibited antioxidant activity.

ETHICAL APPROVAL

All authors declare that handlings of the animals in this study were in accordance with the guidelines published by the National Institute of Health for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 85-23, revised 1985). The research protocol was approved by the Faculty of Pharmacy Ethical Review Committee, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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