

ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITIES OF METHANOL EXTRACT OF *BAPHIA NITIDA*

Abstract-

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

Anti-inflammatory activities of extract was investigated by the carrageenan-induced paw edema model of inflammation, respectively. The antioxidant properties of the extracts as determined by the DPPH free radical scavenging assay. The leaves and root extract demonstrated potent anti-inflammatory activity at low concentrations of between 25-75 mg/kg body weight.

Key words: *Baphia nitida*, anti-oxidant, anti-inflammatory, carrageenan-induced paw edema.

Introduction

At present scenario folk medicine has taken an important role especially in developing countries where limited health services are available. Plant used in treatment of diseases is as old as civilization and traditional medicines are still a major part of habitual treatments of different maladies¹.

Plants are extremely an important source of medicinal agents. However there are different new approaches for drug discovery, such as combinatorial chemistry and computer based molecular modeling design, but none of them can replace the importance of natural products in development and research of medicines².

Inflammation is a complex process, aimed at removing foreign materials such as contaminating micro-organisms and threats from toxins, environmental pollutants, injury, stress and other harmful influences. It is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Inflammation of tissue is due to response to stress³. It is a defensive action that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. It is beneficial and a natural defense mode that works to shield the body's systems and initiate the healing process⁴. 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 and in chronic inflammation they result in irritation and wearing down of cartilages, tissues, and lead to further inflammatory triggers⁵.

Oxidation is a chemical reaction that produces free radicals, leading to chain reactions that may damage cells. An antioxidant inhibits the oxidation of other molecules. Many biological functions, including anti-mutagenicity, anti-carcinogenicity, and anti-aging, may originate from this property⁶. The role of free radicals in human diseases has deepened, antioxidants have attracted broader interest because of their role in inhibiting free radical reactions and their help in protecting the human body against damage by reactive oxygen species. Antioxidants from natural sources have a higher bioavailability and therefore higher protective efficacy comparison of synthetic antioxidants⁷.



Fig 1: *Baphia nitida*

Baphia nitida (Leguminosae- Papilionoideae), has a wide geographical distribution and appears mainly as a shrub or short tree, which grows to a height of about 9 m with immense benefits. 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 altitude⁸. *B. nitida* is popularly called camwood but Yoruba people of West Africa, called it as “Irosun”. Various parts of *B. nitida* have been used by indigenous people of many West African countries for a wide range of medicinal purposes like to treat constipation, ringworm, sprains and swollen joints, parasitic skin diseases, wounds, ulcers, boils, venereal diseases, and gastrointestinal problems and often also used for ornamental purposes⁹. In Nigeria, it is considered anti-inflammatory and therefore used to treat healing wounds, inflamed and infected umbilical cords and to treat sprains and rheumatic complaints¹⁰.

The objective of the present study was to evaluate the anti-inflammatory properties of methanol leaf and root extract of *B. nitida*.

Material and methods

Collection and identification of plant materials

Fresh leaves and roots of *B. nitida* were collected from Ashanti Region of Ghana and authenticated.

Extraction of plant materials

Fresh roots and leaves of *B. nitida* were washed and allowed to dry in air at room temperature (28 to 30°C) for two weeks. The dried leaves were milled into fine powder using the lab mill machine. 2 kg of the sample was percolated in methanol for 48 hrs; this was then filtered and then stored in air tight bottles for analysis¹¹.

Anti-inflammatory studies^{12,13}

Wistar rats (140-190 g) of both sexes were used for the studies. The ethical guidelines for the investigation of animals used in experiments were followed in all tests. The animals were housed in cages under standard laboratory conditions (12:12 hour light/dark cycle at 25 ± 2°C). They had free access to standard commercial diet and water. Three groups A, B and C (comprising of five animals each) of rats were treated orally with 25, 50, and 75 mg/Kg of *B. Nitida* while the control and reference groups received saline (orally) and indomethacin (5 mg/Kg, orally) respectively. Due to painful condition imposed on animals the numbers of subjects used were restricted to the minimum five per group that allowed reliable statistical analysis of the results.

One hour after the administration of extract, indomethacin or saline, 0.1 ml of 1 % carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis.

Measurement of paw size was carried out as described by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. Paw sizes were measured immediately before and 1–5 hrs after carrageenan injection.

Oedema inhibitory activity was calculated according to the following-

$$\% \text{ inhibition} = (T_c - T_t) / T_t \times 100$$

Where, T_c is mean change in paw thickness of control group and T_t is mean changes in paw thickness of treated group.

Table 1: Effects of the methanol extracts of leaves and root of *B. nitida* on carrageenan induced paw edema in rats.

Group	Dose mg/kg	Paw size (mm) ²		% Inhibition	
		3 hr	5 hr	3 hr	5 hr
Control	-	5.1± 0.04	7.3± 0.05	-	-
A	25	5.4± 0.09	2.4± 0.2	36.6	77.1
B	50	3.2± 0.3	1.6± 0.08	61	83.5
C	75	2.7± 0.06	0.7± 0.4	69.4	94.5
Indomethacin	5	5.3± 0.4	1.8± 0.09	30.4	82.5

N = 5, P < 0.05

Anti oxidant study

DPPH antioxidant assay¹⁴

The free radical scavenging activity of *B. nitida* extract was analyzed by, 1,1-diphenyl-2-hydrazyl (DPPH) photometric assay. 2 ml of test extract at concentrations ranging from 10 to 400 µg/ml was each mixed with 1 ml of 0.5 mM DPPH in methanol. Absorbance at 517 nm was taken after 30 min incubation in the dark at room temperature using a spectrophotometer. The experiment was done in triplicates and the percentage antioxidant activity was calculated as follows:

$$\% \text{ of DPPH free radical scavenging} = (\text{Absorbance of (control-blank)}) / (\text{Absorbance of control}) \times 100$$

Where 1 ml methanol and 2 ml extract were used as blank, while 1 ml 0.5 m M DPPH solution and 2 ml methanol was used as control. Ascorbic acid was used as reference standard.

Table 2: Antioxidant activity of the methanol extract of *B. nitida*

Sample	Conc (µg/ml)	Absorbance	Absorbance of control	Mean % DPPH scavenging activity
Ascorbic acid	10	0.079	0.812	87.52 ± 0.56
	50	0.059		90.48 ± 0.09
	100	0.056		91.37 ± 0.82
	200	0.043		92.53 ± 0.63
	400	0.041		94.72 ± 0.84
Methanol extract of <i>B. nitida</i>	10	0.277		63.59 ± 0.68
	50	0.265		72.48 ± 0.42
	100	0.250		74.39 ± 0.54
	200	0.193		77.59 ± 0.71
	400	0.168		81.63 ± 0.38

N=3, p<0.05

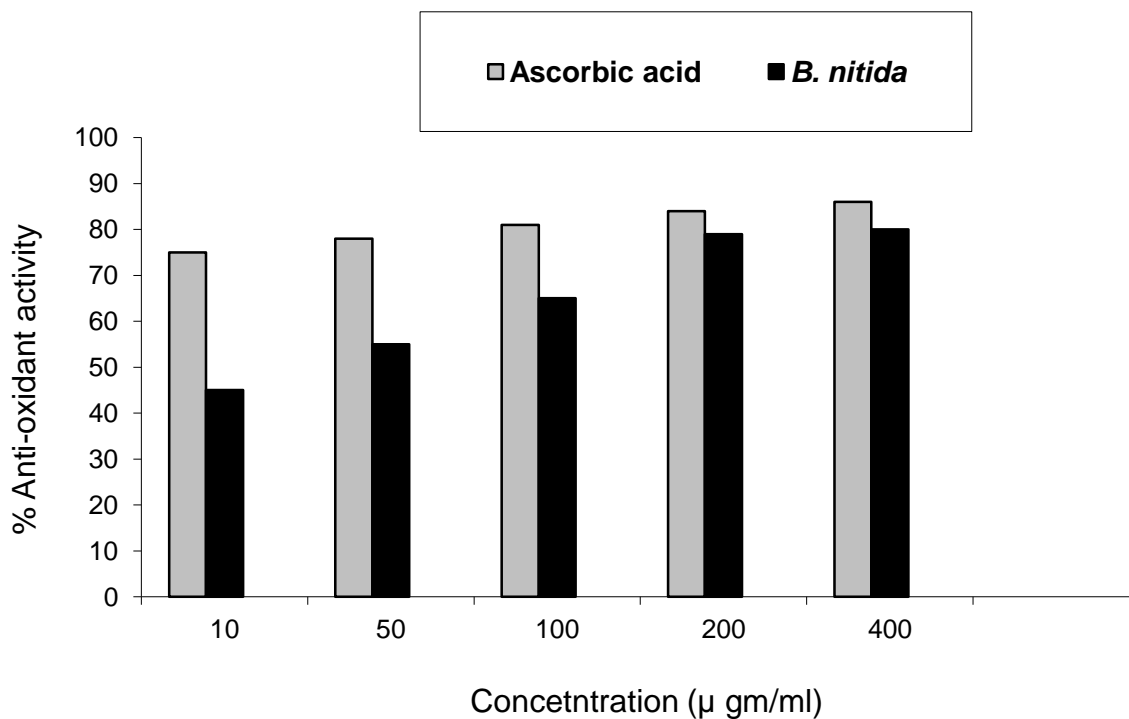


Fig 2: Percentage antioxidant activity of *B. nitida* extract using DPPH photometric assay.

Results and discussion:

The anti-inflammatory activity of a methanolic extract of leaves and root of *B. nitida* was investigated in rats using carrageenan induced paw oedema. Carrageenan-induced rat paw edema model is a popular and widely accepted model for the study of anti-inflammatory activity of compounds which assesses the degree of inflammation and efficacy of test drugs especially at the acute stage. Induction of edema in the paw of rats following subplantar injection of carrageenan results from the release of serotonin, histamine and prostaglandin-like substances¹⁵.

The results show that the extracts significantly ($p < 0.05$) reduced paw oedema dose dependently in the carrageenan test.

The study establishes the anti-inflammatory activity of *B. nitida* leaves.

The antioxidant activity was done in vitro using 1,1-diphenyl-2-hydrazyl (DPPH) spectrophotometric assays. Figure 2 shows the antioxidant activity of *B. nitida* and ascorbic acid standard. The result showed that *B. nitida* extract caused a concentration dependent percentage increase of antioxidant activity. The ascorbic acid standard had a better antioxidant activity of 63.59 % at the concentration of 10 µg/ml and 81.63 % at 400 µg/ml. DPPH is a stable free radical, it accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. The degree of discoloration indicates the radical-scavenging potential of the antioxidant. Result shows that DPPH scavenging was increased in a concentration dependent manner compared to ascorbic acid¹⁶.

Statistical Analysis

Graph Pad Prism Version 5.0 for Windows was used for all statistical analyses. Data are presented as mean \pm SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

Conclusion

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

In the present study, the observed DPPH scavenging activity of the methanolic extracts of root bark might be useful for the development of newer and more potent natural antioxidants. Further phytochemical and pharmacological studies are also required to use their medicinal and pharmaceutical potentialities.

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