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The antioxidant and anti-inflammatory activities of *Picralima nitida* seeds ethanol and hexane extracts

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Abstract

Medicinal plants have been observed to provide cures to certain ailments. Various parts of these plants have been employed ethnomedicinally as remedy for fever, hypertension, jaundice, gastrointestinal disorders, malaria etc. This study evaluated the *in vitro* antioxidant potential and *in vivo* anti-inflammatory activity of the hexane and ethanol extracts of *Picralima nitida* seeds. *In vitro* antioxidant studies carried out include Ferric-reducing antioxidant power assay and DPPH (1, 1-diphenyl-2picryl-hydrdrazyl) radical scavenging activity. The quantitative phytochemical assessment carried out was the total phenol content. *In vivo* anti-inflammatory study was performed using carrageenan induced-paw edema model. Hexane extract and ethanol extract of *P. nitida* seeds possess antioxidant activities whereas ethanol extract possessed higher phenol content than the hexane extract. *In vivo* studies, showed that hexane extract suppressed carrageenan induced paw edema at the late phases of inflammation (3^{rd}-6^{th} hour) when compared with the standard, Diclofenac sodium. This study suggests that *P. nitida* possesses antioxidant and anti-inflammatory properties, with these pharmacological activities among others serving as the base for its use in traditional medicine.

Keywords: *P.nitida,* anti-inflammation, antioxidant, paw edema, carrageenan.

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1. Introduction

Inflammation can be defined as the body's defense reaction to injury in order to eliminate or limit the spread of injurious agents as well as remove consequent necrosed cells and tissues [1]. Inflammation is clinically defined as a pathophysiological process characterized by redness, edema, fever, pain, and loss of function [2]. Antioxidants primarily function to balance out free radicals generated during metabolic processes including during mechanisms involved in protecting the gut from inflammation or injury [3]. *Picralima nitida* is the only species of the genus Picralima and it is related to Hunteria and Pleiocarpa. *P. nitida* is commonly called Picralima, Akuamma or Pile plant; it belongs to the hunterieae tribe of the apocynaceae family. The plant is widely distributed in high deciduous forest of West-Central Africa [4]. The seeds are widely used in Nigeria, as antipyretic, aphrodisiac, for the treatment of malarial and pneumonia [5]. The fruit is used in West Africa for the treatment of gastrointestinal disorder and dysmenorrhea. The leaves are used as an antiparasitic drug and the leaf- sap is extracted and used for the treatment of ear infection. The bark is used in the treatment of venereal diseases and also in the treatment of hernia. Phytochemical screening of *P. nitida* has revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids and glycosides in the plant. Phytochemical investigation has also led to the isolation of a number of alkaloids, which are the major compounds from the seeds of *P. nitida* [6].

2. Materials and Methods

The seeds of *P. nitida* were collected and authenticated from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria with the

voucher number, FHI.112231. The seeds were removed from their husks, dried and blended into fine powder using a mechanical blending machine and stored in airtight containers. 150g of the fine powdery material were macerated into 450 mL of hexane and ethanol (70%) separately for 72 hours at room temperature muslin cloth and Whatman No.1 filter paper and the resulting filtrates were concentrated using a rotary evaporator. The extracts were labeled and stored in the refrigerator for further use.

2.1 Experimental Animals

30 adult male Wistar albino rats were obtained with an initial weight average of 120 ± 20g from the animal facility, Babcock University, Ilishan-Remo, Ogun State. The animals were kept in approved plastic cages, with metal mesh lids and bottoms, at a temperature of 20 ± 2°C and were exposed to a 12-h light dark cycle and left to acclimatized to the new environmental conditions for 7 days. Food and water was supplied throughout the duration of the study. All animals were handled with humane care. This protocol was examined and approved by Babcock University Health Research Ethics Committee (BUHREC), Ilishan-Remo Ogun State, with the ethical approval number BURHEC158/19

2.2 Preparation of test and reference drug solutions

An aqueous solution of carrageenan (1% w/v) was prepared in 0.9% saline and used immediately. *P. nitida* was dissolved in distilled water and the aqueous solution was used for administration. Diclofenac sodium was dissolved in water and used as the standard during administration.

2.3 Anti-inflammatory activity

The effect of *P. nitida* was evaluated on carrageenan induced hind paw edema in rats. Diclofenac sodium was used as a standard drug in the model for comparing the anti-inflammatory potential of *P. nitida*.

2.4 *In vivo* acute model of inflammation

2.4.1 Carrageenan induced paw edema in rats

Thirty albino Wistar rats with an initial weight average of 120±20g were randomly divided into five groups of 6 animals each (n=6);

Group 1: Positive control group received distilled water.

Group 2: Negative control group, received 0.1mL of 1% (w/v) of the carrageenan solution by subcutaneous injection.

Group 3: Standard group received diclofenac sodium 10 mg/kg orally (1 hr prior to carrageenan injection) + 0.1 mL of 1% (w/v) of carrageenan by subcutaneous injection.

Group 4: Received 100mg/kg of *P. nitida* hexane extract orally (1 hr prior to carrageenan injection) + 0.1 mL of 1% (w/v) of carrageenan by subcutaneous injection.

Group 5: Received 200mg/kg of *P. nitida* hexane extract orally (1 hr prior to carrageenan injection) + 0.1 mL of 1% (w/v) of carrageenan by subcutaneous injection.

Groups 3, 4 & 5 received diclofenac (standard), 100mg/kg of *P.nitida* hexane extract and 200mg/kg of *P. nitida* hexane extract 1 h prior to the carrageenan injection. Carrageenan solution (0.1 mL) of 1% w/v was injected subcutaneously into the plantar region of the right hind paw of the rats of the groups (except the positive control group) to produce edema. Paw edema volumes were measured using a micrometer screw gauge at various time intervals at 0, 1, 2, 3, 4, 5 & 6 respectively hr after the carrageenan injection.

2.5 Evaluation of Antioxidant activity

2.5.1 Ferric Reducing Antioxidant Power Assay

The total antioxidant potential of the sample was determined using the Ferric reducing ability of plasma FRAP assay by Benzie *et al.* Method (1996).

For the preparation of the ascorbic acid standard solution, 10 mg of ascorbic acid was dissolved in 100 mL of distilled water. The extract was pipetted in varying concentrations ranging from 50, 100, 200, 250 and 500 µg/mL of the samples. The fresh FRAP reagent was prepared by mixing together 10mM TPTZ (2,4,6-tri (2-pyridyl)-s-triazin) and 20mM ferric chloride in 300mM sodium acetate buffer (pH 3.6). 2 mL The FRAP reagent was mixed with plant extracts and standard solutions. The absorbance was read at 593 nm.

2.5.2 DPPH Radical Scavenging Activity

The ascorbic acid solution was prepared by dissolving 100 mg in 200 mL of distilled water. The extract was pipetted in varying concentrations ranging from 50, 100, 200, 250 and 500 µg/mL of the samples. 1.0 mL of DPPH in methanol (0.3mM) was then pipetted in the standard solution and the extract mixtures. The DPPH reagent was prepared in the dark. The mixture will then be incubated for 30 min in the dark room after which the absorbance will be measured at 517nm. The intensity of the color, measured with the spectrophotometer is inversely proportional to the antioxidant activity of the extracts that we wish to determine the activity. Ascorbic acid was used as standard prepared under the same conditions as the extracts.

The percentage antioxidant potential will be calculated using the formula:

$$\% \text{ Inhibition: } \frac{(absorba\ nce\ of\ control - absorbance\ \ of\ sample}{absorbance\ \ of\ control} \times 100$$

% Inhibition indicates the ability of fractions to inhibit reactive oxygen species.

3. Results

The antioxidant activity of the ethanol and hexane extracts of the seeds of *P. nitida,* and of the mixture of reference (ascorbic acid) with respect to free radical DPPH was evaluated and the results of the reducing activity of extracts (ethanol and hexane) of the studied plant are represented in figure 3.The DPPH radical scavenging activity of extracts and standard increased with decreasing concentration, 83.007% DPPH radical scavenging activity of hexane extract, 98.366% DPPH radical scavenging activity of ascorbic acid standard and 79.521% DPPH radical scavenging activity of ethanol extract at 500 µg/mL. The values indicate that hexane extract exhibited higher antioxidant potential than ethanol extract of *P. nitida* seeds. The administration of P. *nitida* seed extract on Carrageenan Induced-Paw Edema in rat decreases the level of inflammation across the groups as seen in figure 4.

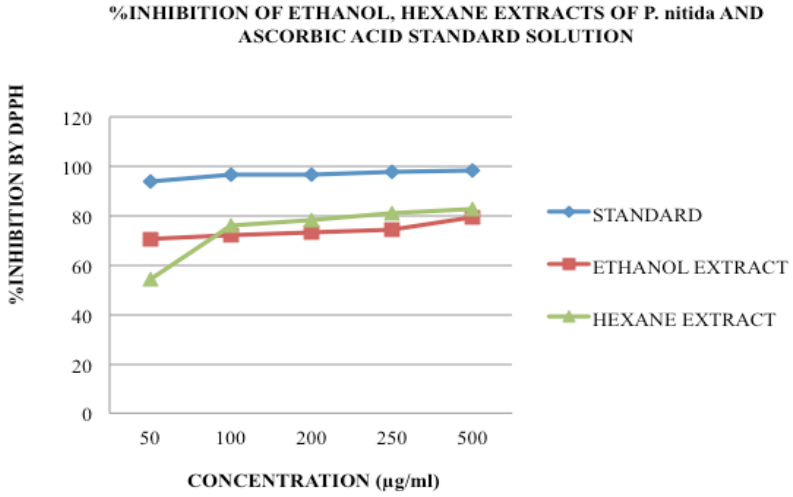

Figure 1: Percentage inhibition of ethanol and hexane extract of *P. nitida* and ascorbic acid standard solution

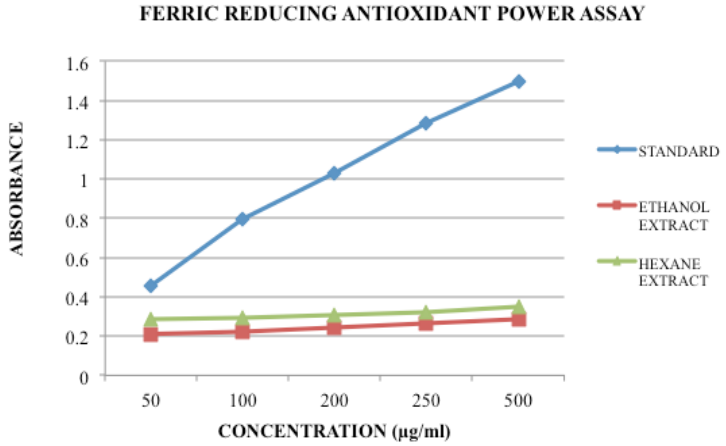

Figure 2: Ferric reducing antioxidant power assay of ethanol and hexane extract of *P. nitida*

This method is based on the principle of increase in the absorbance of the reaction mixtures implying that as absorbance increases, antioxidant activity increases. The data in figure 4 depicts the reductive capacity of aqueous ethanol and hexane extracts in comparison to the ascorbic acid standard. The data in figure 4 shows that hexane extract has higher reductive capacity than ethanol extract, which indicates that the hexane extract has higher antioxidant activity than ethanol extract of *P. nitida* seeds.

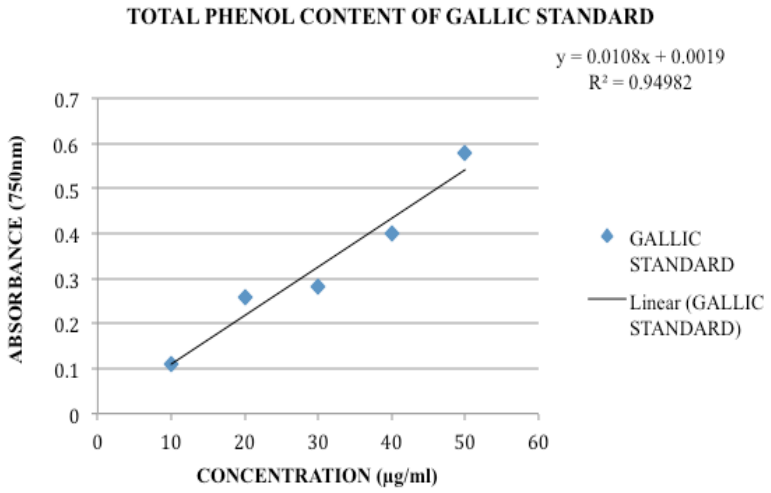

Figure 3: the total phenol content of gallic standard

The total phenol content of the standard (Gallic acid) at different concentrations is shown in the figure 3 above. The crude extracts of *P. nitida* were determined from the equations of the linear regression each calibration curve expressed successively in µg Gallic acid equivalent of per gram of the dry matter.

Table 1: Total phenol content of ethanol and hexane extracts of *P. nitida* in gallic acid equivalent

Extracts	Mean ± SEM
Hexane Extract (100 µg/ml)	7.509 ± 0.008µg/(GAE)/g
Hexane Extract (500 µg/ml)	17.046 ±0.007µg/(GAE)/g
Ethanol Extract (100 µg/ml)	25.380 ±0.003µg/(GAE)/g
Ethanol Extract (500 µg/ml)	35.657 ±0.003µg/(GAE)/g

GAE: Gallic Acid Equivalent

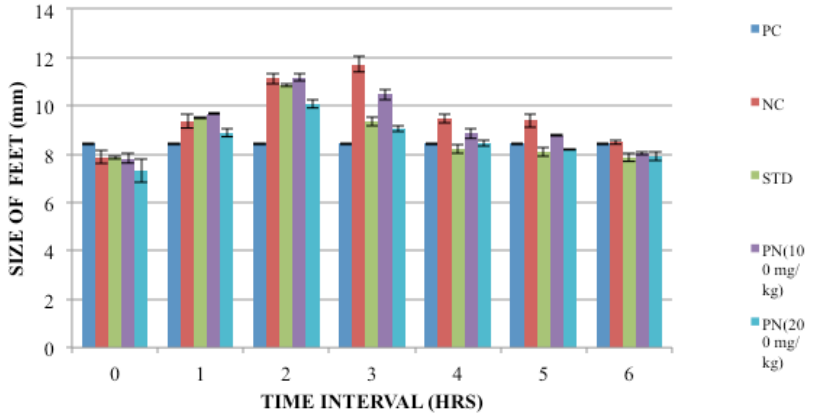

Figure 4: Effect of P. *nitida* hexane extract on Carrageenan Induced-Paw Edema in rats.

(PC: Positive Control, NC: Negative Control, STD: Rat treated with Standard, PN (100mg/kg): P. *nitida* hexane extract (100mg/kg), PN (200mg/kg): *P. nitida* hexane extract (200 mg/kg)).

4. Discussion

Assay of the in *vitro* antioxidant activities in this study revealed that the *P. nitida* extracts possess free radical scavenging activities. The antioxidant activity of the ethanol and hexane extracts of seeds of *P. nitida*, and of the mixture of reference (ascorbic acid) with respect to free radical DPPH was evaluated and the reduction of the DPPH is followed by its passage by the purple color (DPPH·) to the yellow color (DPPH-H). This reduction in capacity is determined by a decrease in absorbance induced by the anti- radical substances. The results of the reducing activity of the ethanol and hexane extracts of the studied plant as represented in figure 2 indicate that *P. nitida* possesses antioxidant activity as compared to a study of the fraction of the aqueous extract of *P. nitida* which presents a significant antioxidant activity comparable to that of the ascorbic acid [7]. Results of ferric-reducing antioxidant power activity (FRAP) showed that hexane extract had slightly higher ferric-reducing antioxidant power than ethanol extract which indicates that the hexane extract has

higher reductive capacity than the aqueous ethanol extract [8]. The hexane extract possibly produced better results than the ethanol extract as a result of the fatty acids present in the seeds which consists of 20.13% saturated and 78.87% unsaturated fatty acids. Also, the seeds contain appreciable percentage of linoleic acid, an essential fatty acid that formed about 33.45% of the total fatty acids in the seeds, which could also account for the results obtained from the hexane extract [9]. The material used in the present study to produce acute inflammation is carrageenan, which is a sulphated polysaccharide obtained from red green algae (Rhodophyceae).

Carrageenan induced rat paw edema has been described as a biphasic event in which various mediators operate in sequence to produce the inflammatory response [10]. From Figure 4, at the late phases of inflammation (3^{rd}-6^{th} hours), there was inhibition of paw edema by *P. nitida* hexane extract at 100mg/kg and 200mg/kg when compared to the standard group. This indicates that the *P. nitida* hexane extract possesses in *vivo* anti-inflammatory activity perhaps by inhibiting the release of the inflammatory mediators, serotonin and histamine or also by suppressing prostaglandin and cytokine synthesis.

5. Conclusion

Findings from this study indicate that *P. nitida* possesses antioxidant activity. However hexane extract demonstrated higher antioxidant potential than ethanol extract. Also, hexane extract of *P. nitida* seeds showed anti-inflammatory activity in Carrageenan-induced paw edema, which could be by inhibiting synthesis of proinflammatory mediators. Further studies should be done to determine the bioactive components present in *P. nitida* seeds. Also, more *in vitro* and *in vivo* studies of non-polar fractions of *P. nitida* seeds should be further explored to ascertain its clinical use.

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