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Inhibitory Effects of *Bryophyllum pinnatum* Leaf Extract on Inflammatory Biomarkers

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OEI and OHA managed the analyses of the study. Authors OCS and OCU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Bryophyllum pinnatum Lam. (Crassulaceae) called '*Oda-opue*' in Igbo and '*Abamoda*' in Yoruba are widely used as food and as medicines in traditional medical practice. They are found widely in tropical Africa, America, India and China. This study investigated the inhibitory effect of the hydro-ethanol leaf extract on inflammatory biomarkers. The Cotton Pellet Induced Granuloma method was used in the study. The plant extract significantly inhibited the inflammatory biomarkers, cyclooxygenases 1 and 2, interleukins 1β and 6, and prostaglandin E2, in a dose dependent manner indicating a reduction of inflammation in the rats. The study showed that *B. pinnatum* leaf extract possess a rich content of bioactive compounds which could be synthesized to produce new plant-based product to fight inflammatory disorders with fewer side effects.

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Keywords: Bryophyllum pinnatum; inflammation; biomarkers; chronic diseases; human health.

1. INTRODUCTION

Bryophyllum pinnatum (Lam) Oken (Crassulacea) is a tropical, perennial herb that grows widely in tropical Africa, America and Asia. The succulent herb is known for its numerous beneficial activities such as anti-leshammanial activity as well as hepato-protective and nephro-protective effect [1], particularly its curative effect on chronic inflammatory diseases and wound healing properties [2]. The plant is broadly used locally to treat several illnesses such as external ulcers, syphilis, candidiasis, convulsion, jaundice and burns [3].

Inflammation is a dynamic process that is elicited in response to mechanical injuries, burns, microbial infections and other noxious stimuli that may threaten the well-being of the host [4]. This process could be self-limiting (acute) or persistent and prolonged (chronic). The chronic form is known to be the highest cause of mortality globally today, with over 50% of total deaths traceable to inflammation-related diseases such as asthma, chronic peptic ulcer, tuberculosis, sinusitis and active hepatitis [5]. Researches have revealed that identification and manipulation of pro-inflammatory cytokines may check or mitigate the destructive effect on tissues and prevent progression to chronic diseases [6,7].

The continent of Africa is blessed with plethora of medicinal plants. Majority of these plants possess anti-inflammatory activity and therefore serve as first contact in meeting the primary health care needs of the people by reason of the accessibility, affordability, cultural and spiritual acceptance, and the knowledge of its preparation and use.

Nowadays, many anti-inflammatory drugs are available everywhere. However, most of these drugs, especially the non-steroidal anti - inflammatory drugs (NSAIDs), have been reported to be deleterious owing to their various side effects [8]. On the other hand, medicinal plants have been reported to have fewer or no side effects [9]. This has led to an exhaustive search for anti-inflammatory drugs with fewer side effects. This study, therefore, investigated the inhibitory effect of *B. pinnatum* leaf extract on inflammatory biomarkers in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material

Fresh green leaves of *B. pinnatum* were collected from International Center for Ethno-medicine and Drug Development (InterCEDD), Nsukka, Enugu-State, Nigeria. Identification and authentication of the plant was carried out by a taxonomist, Mr. A. O. Ozioko, of InterCEDD and a voucher specimen was deposited at the InterCEDD herbarium (specimen number: BDCP/INTERCEDD-78). The plant material was shredded with a knife and air-dried under shade for 21 days.

2.2 Extraction of Plant Materials

The dried plant (leaves) was pulverized using a laboratory grinder and the fine powder obtained was stored in an air tight container at room temperature until further use. Weighed powdered sample was extracted with 70% ethanol (by maceration) for 72 hours. The yield of extracts was calculated according to the method of Nkafamiya et al. [10] using the formula below:

> Percentage yield = Mass of Extract after rotary evaporation (g) /Mass of crude extract (g) x 100

2.3 Determination of Median Lethal Dose (Ld_{50})

The median lethal dose was determined using Lorke's method [11].

2.4 Experimental Animals

Wistar albino rats (30) weighing between 180 g - 250 g were obtained from Chris Farm Ltd Mgbakwu, Awka, Anambra State. They were sorted, housed in standard cages with housing conditions of 12:12 light: dark cycles. They were fed with standard rat pellet and water *ad libitum*. All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Laboratory Animal Care of the National Society of Medical Research [12].

2.5 Dose Preparation and Treatment

The hydro-ethanolic leaf extract of *B. pinnatum* was prepared with distilled water in three divided dose (100, 200, and 400) mg / kg,

Dexamethasone (25 mg/kg) used as a reference drug, distilled water as untreated group. The animals were administered the extract and drug for seven consecutive days with water *per os* and feed *ad libitum* [13].

2.6 Induction of Inflammation

Cotton Pellet was used to induce chronic inflammation (granuloma) in the animals. One sterile cotton pellet weighing 20 mg each was implanted subcutaneously into the groin region of each anaesthetized rat.

2.7 Collection of Blood Sample and Assay of Inflammatory Biomarkers

At the end seventh day, the experimental animals were anaesthetized with chloroform vapor, and sacrificed. A 5ml sterile syringe with needle was used for collection of blood via cardiac puncture and was used for bioassay studies. Bioassay of Cyclooxygenase 1 and 2, interleukins 1 and 6 and prostaglandin E_2 were all carried out using standard ELISA assay kit sourced from RANDOX Laboratories Ltd., Crumlin, Co. Antrim, UK.

2.8 Data Analysis

The results were expressed as Mean ± S.E.M. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$.The graphical analyses were carried out using Graph-Pad Prism5 Program (Graph-Pad Software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

3.1 Toxicological Studies

The LD_{50} was 2154 mg/kg body weight; it was calculated as shown below:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

$LD_{50} = \sqrt{(1600 \times 2900)} = 2154$ mg/kg body weight

3.1 Inhibitory Effects of *B. pinnatum*

The effects of *B pinnatum* leaf extract on enzymes involved in inflammatory pathways in the experimental animals are shown in Fig. 1–5. The enzymes are cyclooxygenase-1, cyclooxygenase-2, Interleukin-1, interlukin-6, and Prostaglandin E_2.

Group A: Received 100 mg/kg dose of extract of *B. pinnatum* leaf extract.
Group B: Received 200 mg/kg dose of extract of *B. pinnatum* leaf extract.
Group C: Received 400 mg/kg dose of extract of *B. pinnatum* leaf extract.
Group D: Received 25 mg/kg dose of Dexamethasone tablet
Group E: Served as negative control and received distilled water.

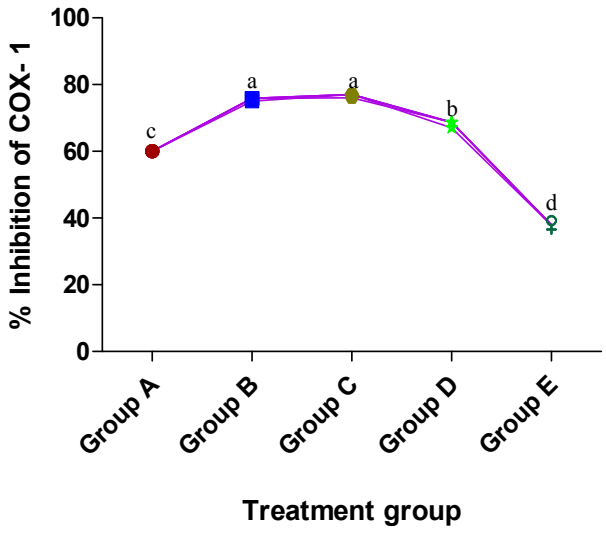

Fig. 1. Effect of *B. pinnatum* leaf extract on Cyclooxygenase-1 activity
Colums with different alphabets are significantly different at P< 0.05

The leaf extract of *B. pinnatum* was found to exert inhibitory effect on cyclooxygenase-1 (COX-1) activity in Groups B and C animals (Fig. 1). The response of the animals to treatment was dose dependent (100, 200, and 400) mg/kg body weight, with significant ($p \leq 0.05$) inhibition in the COX-1 activity. The ethanol extract of *B. pinnatum* showed an inhibitory effect on COX – 1 activity. This result is consistent with the findings of Khan et al. [14]. This observation is noteworthy, as cyclooxygenase (COX) inhibitors are among the most commonly used drugs in the world for their anti-inflammatory and analgesic properties [14]. COX-1 is the enzyme that catalyzes the key step in the conversion of arachidonate prostaglandin-2 (PGH-2), the immediate substrate of all specific prostaglandin and thromboxane synthases. The constitutively expressed COX-1 is present in cells under physiological condition and produces protective substances for the stomach and kidney [15]. The reduction in activity observed in this study indicates decreased production of pro-inflammatory mediators [16]. COX-1 is responsible for the production of prostanoids that maintain mucosal blood flow and promote mucous secretion [17]. Its activation leads to production of prostacyclin which when released by the vascular endothelium is anti-thrombogenic [18], and when released by gastric mucosa is cytoprotective [18].

Fig. 2 presents the effect of *B. pinnatum* leaf extract on cyclooxygenase-2 activity in the experimental animals. The response of experimental animals to treatment was dose dependent (100 mg/kg, 200 mg/kg, 400 mg/kg body weight). There was a significant inhibition of COX-2 enzyme in group C animals. Group B and D shows no significant difference between each other. However, when compared to group E animals, there is significant difference at $p < 0.05$.

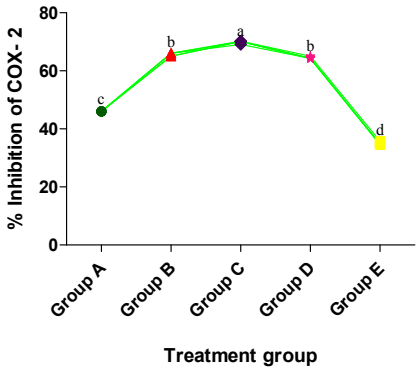

Fig. 2. Effect of *B. pinnatum* leaf extract on Cyclooxygenase-2 activity
Colums with different alphabets are significantly different at P< 0.05

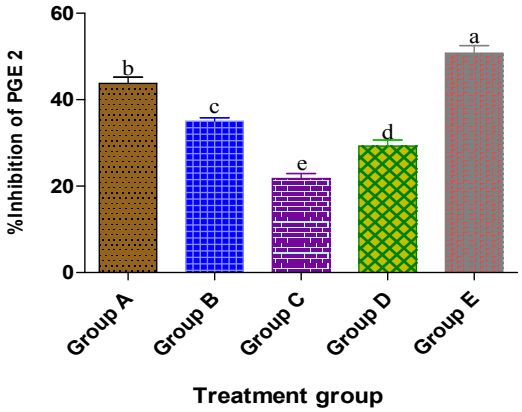

Fig. 3. Effect of *B. pinnatum* leaf extract on Prostaglandin E_2
Colums with different alphabets are significantly different at P< 0.05

This result agrees with the reports of Elisabetta et al. [19]. COX-2 is the enzyme known to aid the production of inflammatory mediators' prostaglandin (PG) and its metabolites such as prostaglandin E_2, prostaglandin F_2 and prostaglandin D_2. It is synthesized by macrophages and induced by tumor necrosis factor (TNF) α and epidermal growth factor (EGF). COX-2 is also known as prostaglandin-endoperoxidase synthase and belongs to a family of isoenzymes responsible for the formation of prostanoids including thromboxane and prostacyclin from arachidonic acid.

Another notable inflammatory biomarker that is of concern is the prostaglandin E_2 (PGE_2). It is a group of physiologically active lipid compound called dinoprostone derived enzymatically from arachidonic acid that has several hormone-like effects in animals. It is released by blood vessel walls in response to infection or an inflammation, which induces fever and participates in a wide range of body functions such as contraction and relaxation of smooth muscle, dilation and constriction of blood vessels, and modulation of inflammation [20].

Fig. 4 presents the effect of *B. pinnatum* leaf extract on Interleukin-1β activity in the experimental animals. The response of experimental animals to treatment was dose dependent (100 mg/kg, 200 mg/kg, 400 mg/kg body weight). There was a significant inhibition of Interleukin-1β in all the experimental groups at $p< 0.05$ with highest inhibition observed in group C animals.

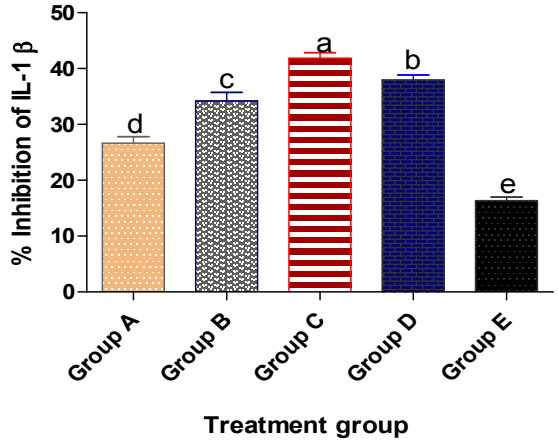

Fig. 4. Effect of *B. pinnatum* leaf extract on Interleukin-1β activity
Colums with different alphabets are significantly different at P< 0.05

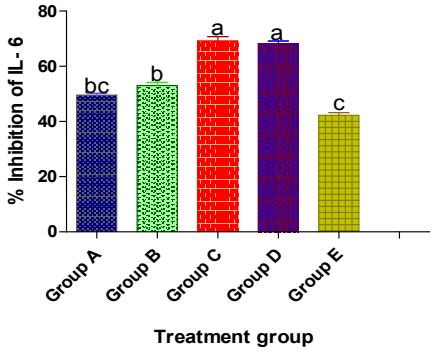

Fig. 5. Effect of *B. pinnatum* leaf extract on Interleukin-6 activity
Colums with different alphabets are significantly different at P< 0.05

Fig. 5 presents the effect of *B. pinnatum* leaf extract on Interleukin-6 activity in the experimental animals. The response of experimental animals to treatment was dose dependent (100 mg/kg, 200 mg/kg, 400 mg/kg body weight). There was a significant inhibition of Interleukin-6 in groups C and D animals. Groups A and B showed slight difference with significant difference when compared with group E animals at $p< 0.05$.

The inhibitory effect of the extract on Interleukin -1β (IL-1β) and Interleukin 6 (IL-6) is shown in Fig. 4 and 5. Interleukin (IL-1β) is a primary mediator of inflammatory response and is implicated in many cellular activities including cell differentiation and apoptosis [21]. Researches have shown that they induce COX-1 and TNF-α and produce notably active antibodies but their pathophysiology is not well known yet [21].

Interleukin 6 (IL-6) is involved in various physiological functions including hematopoiesis, neuro-development and bone metabolism [21] it is involved in many inflammatory diseases such as arthritis, osteoporosis and diabetes [22]. IL-6 is a multi-functional cytokine that is important in host defense and immune reactions [23], and its increased level has been linked to various pathological conditions such as bacterial and viral infections and inflammation [24]. Hence, regulation of IL-6 might be effective against various diseases [25].

4. CONCLUSION

The plant extract caused a significant inhibition of inflammatory biomarkers (cyclooxygenases 1 and 2, interleukins 1β and 6 as well as prostaglandin E-2) in a dose dependent manner. Hence, the ability of the plant extract to inhibit the precursors of these prostaglandins could be a possible way to explain its mechanism of anti-inflammatory action.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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