



## **Acute and Chronic Anti-inflammatory effect of *Bryophyllum pinnatum* Leaf Extract on Wistar Rats**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### **ABSTRACT**

*Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) is used traditionally to treat many ailments. This study investigated the anti-inflammatory effect of hydro-ethanol leaf extract of *Bryophyllum pinnatum* on Wistar rats using acute and chronic models and also evaluates the bioactive compounds of the leaf extract. The phytochemical constituents of the plant extract were quantitatively determined by Gas Chromatography-Flame Ionization Detector (GC-FID) and acute anti-inflammatory activity was carried out with the aid of plethysmometer while chronic anti-inflammatory activity was investigated using cotton pellet. Results showed that the leaf extract of *B. pinnatum* was rich in kaempferol ( $7.006 \pm 0.02 \mu\text{g/g}$ ), sapogenin ( $3.372 \pm 0.02 \mu\text{g/g}$ ), rutin ( $1.837 \pm 0.01 \mu\text{g/g}$ ) and lunamarine ( $1.359 \pm 0.01 \mu\text{g/g}$ ). The findings showed that the plant had considerable anti-inflammatory effects in a dose dependent manner, returning edema in carrageenan-induced and cotton pellet induced granuloma in Wistar rats to normal within 120 minutes and 7 days respectively. The findings of this work have shown that the leaf of *B. pinnatum* was rich in bioactive compounds which could be synthesized to produce new plant based product to fight inflammatory disorders with fewer side effects.

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## 1. INTRODUCTION

For centuries, inflammation has been known to be the underlying pathophysiological mechanisms of many clinical disorders [1,2]. It could be acute or chronic in occurrence and each is associated with a wide range of body disorders or discomfort. Inflammation is triggered by many noxious stimuli which include infections (viral, bacterial and fungal), chemical/physical agents, defective immune system and trauma and these could clinically present as pain, resulting in injuries to the tissues or body system. Classical inflammatory response involves macrophages and neutrophils and these are known to secrete various mediators that are known to promote and perpetuate inflammation [3]. The sole aim of these reactions is to protect and wade off the offending stimuli, to remove debris and to repair the affected tissue. Where the resolution could not completely occur in acute phase, the reaction proceeds to the chronic stage. The chronic form of inflammation has been known to be the bedrock of many chronic diseases including various forms of cardiovascular disorders and many rheumatic and immune mediated conditions [2, 4].

Non-steroidal anti-inflammatory agents have been found useful in the management of most chronic inflammatory conditions. These chemical formulated drugs have been in use for many decades and these have many potential drawbacks, including high cost, non-availability locally and potentially many side effects or adverse drug reactions [5].

Many plants possess medicinal anti-inflammatory properties that have been found useful in the management of these conditions in many traditional settings in most tropical and sub-tropical countries [6, 7]. They have the special advantage of being cheap, locally available and grossly devoid of many side effects associated with chemical drugs [8]. The African continent is richly endowed with such medical anti-inflammatory plants that have been found useful in the traditional medical practice setting.

Findings from traditional medicine practitioners on some plants showed that *Bryophyllum pinnatum* (Crussulaceae) is one of the most promising plants which could help to ameliorate

inflammatory diseases that pose great health challenge to human worldwide.

*B. pinnatum* is a herb that grows in the wild and used as a traditional medicine as well as ornamental plant in tropical Africa, China, Australia, tropical America and India [9]. The plant is a perennial herb with hollow stem. It is 1-1.5 meters high and does not have branches. The leaves appear to be the most valuable part of the plant for medicinal purposes [9]. Thus, this study evaluates the anti-inflammatory effect of *Bryophyllum pinnatum* leaf extract using both acute and chronic inflammatory models.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

Fresh green leaves of *B. pinnatum* were collected from International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu-State, Nigeria. Identification and authentication of the plant was carried out by 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 42 days. The dried leaf was pulverized using a laboratory blender and the fine powders obtained was weighed and stored in an air-tight container at room temperature until further use.

### 2.2 Extraction of Plant Materials

The weighed powdered sample (500 g) was then used for the extraction with solvent combination of water and ethanol (3:7) (3500 ml) for 72 hr. The yield of extract (15 g) was calculated according to the method of Nkafamiya et al [10] using the formula below:

$$\text{Percentage yield} = \frac{\text{mass of extract (g)}}{\text{mass of sample (g)}} \times 100$$

### 2.3 Quantification (GC-FID) of Phyto - chemical Content of the Plant Extract

The method of Kelly and Nelson [11] was used for the quantification of the phytochemical content. The analysis of free steroids was performed on a BUCK M910 Gas chromat-

graphy equipped with a flame ionization detector and the concentrations of the different phytochemicals were expressed in µg/g.

## 2.4 Procurement of Study Animals

Thirty (30) adult Wistar albino rats of both sexes weighing 150-200 g were purchased from Chris farms, Mgbakwu town, Awka north, Anambra State. The rats were kept in standard cages with saw dust as bedding and standard housing condition and fed with standard rat pellets and water *ad libitum*. All the experimental procedures and protocols used in this study were in accordance with the guidelines principles of Laboratory Animal Care of the National Society of Medical Research (NIH Publication; Guide for the care and use of laboratory animals, No. 85-23, revised 1985).

### 2.4.1 Animal grouping and dose administration

The rats were separated into five (5) groups (A-E) of six (6) rats per group, per cage. The plant extract was prepared in distilled water (5 g in 100 ml) at three divided dose (100 mg/kg, 200 mg/kg and 400 mg/kg) and Dexamethasone (25 mg/kg) as shown in the Table below.

**Table 1. Grouping and dose administration for animals**

Group	Treatment
A	Oedema plus 100 mg/kg extract sample
B	Oedema plus 200 mg/kg extract sample
C	Oedema plus 400 mg/kg extract sample
D	Oedema plus 25 mg/kg standard drug
E	Oedema without treatment

## 2.5 Place of Study

The research was carried out at the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State Nigeria.

## 2.6 Rat Paw Oedema Induction Using Carrageenan Principle

Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic

agent for the choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect. Moreover, the model exhibits a high degree of reproducibility. The probable mechanism of action of carrageenan-induced inflammation is bi-phasic; the first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substance which peak at 3 hours.

### 2.6.1 Method

Acute anti-inflammatory study was carried out according to the method of Winter et al[12].

### 2.6.2 Procedure

Acute inflammation was produced by sub plantar injection of 0.1ml of 1% suspension of carrageenan in normal saline in the right hind paw of the rats 60 minutes after the oral administration of extracts and vehicles. The paw volume was measured by digital Plethysmometer at 0 minutes, 30 minutes, 60 minutes, and 120 minutes after the induction of inflammation on the same day. The percentage inhibition of oedema with that of control was taken as anti-inflammatory activity. The percentage inhibition of oedema was calculated by the formular:

$$\text{Percentage inhibition of oedema} = \frac{(A-B)}{A} \times 100$$

Where A represents the paw volume of control group and B represents the paw volume of treated group.

## 2.7 Cotton Pellet Induced Granuloma Principle

This is an established animal model to screen the chronic anti-inflammatory activity of a test compound. It assesses the transudative, exudative and proliferative phase of chronic inflammation.

### 2.7.1 Method

The method of Goldstein et al[13] was used for chronic anti-inflammatory activity.

### 2.7.2 Procedure

One sterile cotton pellet weighing 20 mg each was implanted subcutaneously in anaesthetized animals in one groin region of each rat. Vehicle, standard drug and plant samples (100mg, 200mg and 400mg) were administered for seven

consecutive days with free access to water and food *ad libitum*. On the eighth day, the animals were sacrificed by excessive anaesthesia and the cotton pellets were removed surgically, cleaned of the extraneous tissue and dried in a hot air oven at 60<sup>o</sup> C to a constant weight and the dry weight of pellets were determined. The dry weight of the granuloma was calculated by noting the difference in the dry weight of the cotton pellets recorded before and after implantation. The percentage change of granuloma weight relative to control group was taken as an index of chronic anti-inflammatory activity. The percent inhibition increase in the weight of cotton pellets was calculated by:

$$\% \text{ inhibition} = \frac{(X-Y)}{X} \times 100$$

Where X is the pellet weight of the control group and Y is the pellet weight of the treated group.

## 2.8 Sacrifice and Sample Collection

On the eight day, the experimental animals were all anaesthetized with chloroform and blood was drawn slowly through heart puncture. Blood samples were dispersed into ethylenediaminetetracetic acid (EDTA) bottles (for hematological analysis) and universal bottles (for enzyme bioassay). The samples in the universal bottles were allowed to clot, after which they were centrifuged for 10 minutes at 4000 rpm. The sera obtained were transferred into another bottle for further bioassay studies.

## 2.9 Data Analysis

The results were expressed as Mean  $\pm$  S.E.M. One way analysis of variance (ANOVA) was carried out on both the *in vitro* and *in vivo* results and significance was accepted at  $p < 0.05$ . The graphical analyses were carried out using GraphPad Prism5 Program (GraphPad Software, San Diego, CA, USA).

## 3. RESULTS AND DISCUSSION

Plants play a vital role in discoveries associated with new beneficial therapeutic agents and have received significant focus because of their bio-active substances. Plants have invariably been exemplary source of drugs and a number of currently available drugs happen to be derived directly or indirectly from them.

Phytochemical analysis is very useful in the evaluation of some active biological compounds of some medicinal plants. The quantitative phytochemical analysis of the leaves of *Bryophyllum pinnatum* were carried out and kaempferol, spartein, anthocyanin, oxalate, sapogenin, rutin, lunamarine, ribalinidine, phytate, catechin and flavonoid were found to be present in the plant samples. Kaempferol was present in appreciable amount (7.006  $\pm$  0.02  $\mu$ g/g) (Table 2). This is consistent with the report of Barve et al [14]. Kaempferol is a natural flavonol, yellow crystalline solid, and highly soluble in ethanol. It is known to reduce the risk of chronic diseases, especially cancer [15]. It has been shown to augment human body's antioxidant defense against free radicals [16], and modulates apoptosis, angiogenesis, inflammation and metastasis [17].

The sapogenin content (3.372  $\pm$  0.00  $\mu$ g/g) (Table 2) in the plant samples implies that the leaves of *Bryophyllum pinnatum* can help to decrease blood lipids, lower cancer risks and lower blood glucose response [18]. Clinical studies have suggested that these health-promoting components affect the immune systems in ways that help to protect the human body against cancers and also lower cholesterol levels [19].

Rutin is another compound that was present in an appreciable quantity (1.837  $\pm$  0.01  $\mu$ g/g) (Table 2). Rutin is a bioflavonoid with powerful antioxidant properties [20]. Rutin has been shown to reduce body weight by 7.9 % [21], improve eye health by strengthening fragile capillaries [22], and as well possess anti-inflammatory effects [23].

Other compounds such as spartein (0.005  $\pm$  0.00  $\mu$ g/g), ribalinidine (0.027  $\pm$  0.00  $\mu$ g/g) and anthocyanin (0.097  $\pm$  0.01  $\mu$ g/g) were present in minute quantities. This is consistent with the findings of Ogidigo et al [24].

Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent for the choice of testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect. Moreover, the model exhibits a high degree of reproducibility.

**Table 2. Phytochemical constituents of ethanol leaf extract of *B. pinnatum***

Component	Concentration ( $\mu\text{g/g}$ )
Kaempferol	7.006 $\pm$ 0.02
Sparteine	0.005 $\pm$ 0.00
Anthocyanin	0.097 $\pm$ 0.01
Oxalate	0.196 $\pm$ 0.03
Sapogenin	3.372 $\pm$ 0.00
Rutin	1.837 $\pm$ 0.00
Lunamarine	1.359 $\pm$ 0.01
Ribalinidine	0.027 $\pm$ 0.00
Phytate	0.234 $\pm$ 0.03
Catechin	0.235 $\pm$ 0.03
Total flavonoid	0.276 $\pm$ 0.02

Values are means  $\pm$  standard error of mean.

As shown in Fig 1, administration of Carrageenan in sub plantar region of left hind paw significantly increased the paw volume. However, oral administration of ethanol leaf extract of *B. pinnatum* produced significant oedema inhibitory effect in rats by reducing paw volume at a dose dependent manner (100 mg/kg, 200 mg/kg and 400 mg/kg) body weight after 30, 60 and 120 minutes when compared with the control group. As evident in Fig. 1, percentage inhibition of paw oedema was found to be 35.5 % at 400 mg/kg after 120 minutes. Dexamethasone produced a significant anti-inflammatory activity (40 % inhibition of paw edema) with respect to inflammation control. This result is consistent with the findings of Suresha et al [25] and Pandurangan et al [26] where the authors in separate studies recorded oedema inhibition of 30.8 % and 43.37 % for Cetrizine and chloroform extract of *B. calycinum* plant respectively, but lower to 92.73 % edema inhibition from *Hamelia patens* leaf extract reported by Vijay-Raj et al [27]. Since the induction of Carrageenan is characterized with the release of histamine, serotonin and kinins; and histamine has been reported to be an organic nitrogenous compound that are involved in local immune and inflammatory responses with a central role as a mediator of itching, while serotonin is said to be a happy chemical and therefore considered as natural mood stabilizer., the extract of *B. pinnatum* might have probably worked via suppressing the release of these endogenous substances and inflammatory mediators to produce peripheral anti-inflammatory activity.

Cotton pellet induced granuloma is an established animal model to screen the chronic

anti-inflammatory activity of drugs. As depicted in Fig. 2, implantation of cotton pellet in the

experimental animals caused a significant inhibition of induced granuloma at the incised area in a dose dependent manner (100 mg/kg, 200 mg/kg and 400 mg/kg) body weight. The percentage inhibitions of granuloma formation were 42.02 %, 48.76 %, and 58.24 % respectively (Fig. 2). This result is consistent with the findings of Sultana et al [28] where the authors reported the percentage inhibition of granuloma formation by *Carica papaya* to be 49.06%, 53.35% and 62.51% at 100 mg/kg, 200 mg/kg and 400 mg/kg body weight respectively as well as the reported 39.1 % inhibition of granuloma by Cetrizine as documented by Suresha et al [25].

The anti-inflammatory activity of the plant could be traced to its flavonoids and alkaloid content. A number of studies recorded that several plant extracts showed anti-inflammatory effect in animal models and their effects have been attributed to the presence of flavonoids, alkaloids triterpenoids, glycosides, tannins and sterols [29,30]. Flavonoids and alkaloids are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [31]. Flavonoids also inhibit inflammatory processes by inhibiting phosphodiesterases that are involved in cell activation [32].

In the same vein, flavonoids are involved in induction of endogenous serotonin secretion or interaction with 5-HT<sub>2A</sub> and 5-HT<sub>3</sub> receptors involved as a probable mechanism of central anti-inflammatory activity [33]. On the other hand, various studies reported that alkaloids isolated from various plants have shown inhibitory effect on eosinophil recruitment, leukotriene production

in the pleural cavities, as well as inhibiting the production of nitric oxide mediators which result in anti-inflammatory effect [34]. In addition, polyphenols exert their anti-inflammatory properties through inhibition of the production of inflammatory cytokines and chemokines and suppressing the activity of cyclo-oxygenase (COX) and inducible nitric oxide synthase (iNOS) and thereby decreasing the production of reactive oxygen and nitrogen species (ROS/RNS) [35]. Thus, it is possible that at least part of the anti-inflammatory activity showed in this study by the ethanol extracts of *B. pinnatum* may be due to the presence of these polyphenolic substances.

The anti-inflammatory effect of *B. pinnatum* could also be hypothesized to be due to its effect on human neutrophils and its ability to suppress the expression of macrophage migratory inhibitory factor. Neutrophils release cytokines including IL-1, IL-6, TNF- $\alpha$ , interferon  $\gamma$  and others. Such pro-inflammatory cytokines in turn induce the liver to synthesize various acute phase reactant proteins and also produce systemic inflammatory responses like fever and leukocytosis. The other possible mechanism of its anti-inflammatory action could be possibly due to inhibition of bradykinin, ICAM-1 expression [36] and PGE<sub>2</sub> activation [30,37].

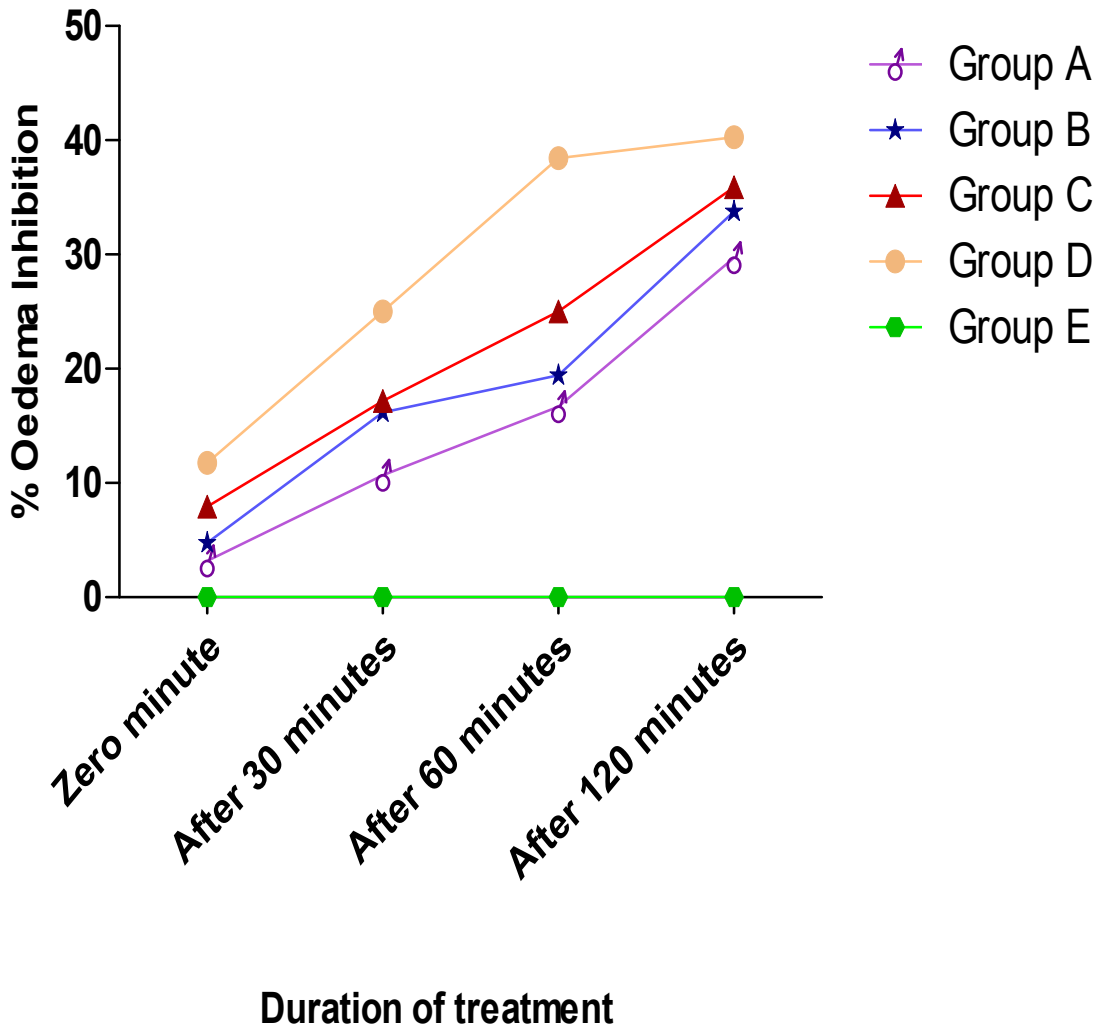
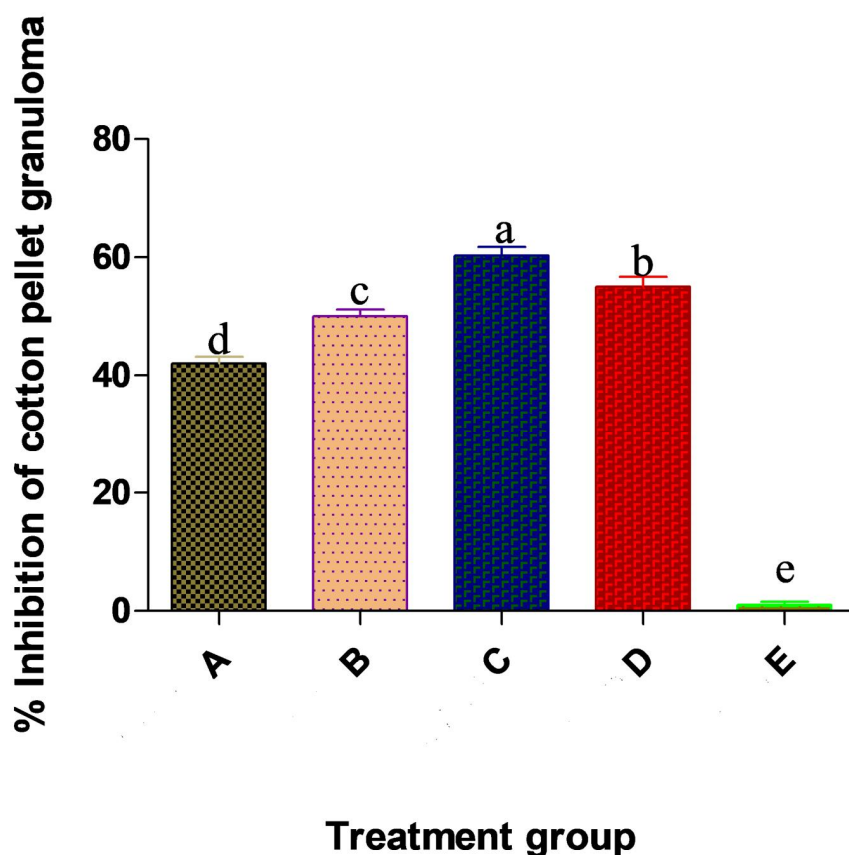


Fig. 1. Effect of *B. pinnatum* leaf extract on Oedema inhibition after 120 minutes



**Fig. 2. Effect of *B. pinnatum* leaf extract on cotton pellet granuloma inhibition after seven days**  
Columns with different alphabets are significantly different at  $P < 0.05$

#### 4. CONCLUSION

The observations from the present study showed that the plant extract contained pharmaceutically significant bioactive compounds which could help to ameliorate various chronic disorders, especially inflammation. The plant showed a significant inhibition of paw edema and inhibition of induced granuloma with respect to inflammation. The anti-inflammatory effect of *B. pinnatum* leave extract could be attributed to many bioactive compounds as revealed by GC-FID which could be synthesized to produce new plant based product to fight inflammatory disorders with fewer side effects.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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