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The Effect of *Bryophyllum Pinnatum* (*Lam*) *Oken* (*Crassulaceae*) Extract on Enzymes Involved in the Inflammatory Pathway

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Abstract

Bryophyllum pinnatum Lam.Oken (Crassulaceae) is widely used as food and as medicine in traditional medicine. The plant is found widely in tropical Africa, America, India and China. This study explores the bioactive compounds in the plant and investigates the effect of the hydro ethanol leaf extract on enzymes involved in the inflammatory pathways using GCMS and Cotton Pellet Induced Granuloma method. Results revealed that the hydro-ethanol leaf extract showed nine peaks, indicating nine bioactive compounds with 2-Amino-3-hydroxypyridine being highest (34.678%). Our findings indicated that the plant's extract reduced inflammation in rats, causing a significant inhibition of inflammatory biomarkers (cyclooxygenases 1 and 2, interleukins 1β and 6 as well as prostaglandin E2) in a dose dependent manner. *B. pinnatum* leaf extract showed rich content of bioactive compounds which could be synthesized to produce new plant-based product to fight inflammatory disorders with fewer side effects.

Keywords: B. pinnatum • Bioactive components • Inflammation • Chronic diseases • Human health

Introduction

The plant *Bryophyllum pinnatum* (Lam)Oken (Crassulacea) has been reported to possess numerous activitiese specially its ameliorative effect on chronic inflammatory diseases It is a tropical, perennial herb that grows in Africa and Asia [1]. It is a succulent herb widely used in traditional medicine for treatment of many ailments such as syphilis, candidiasis, jaundice, external ulcers, burns, and convulsion.

Inflammation is a naturally conserved process of the body's response to a wide variety of stimulus and it is characterized by the activation of immune and non-immune cells that protect the host from bacteria, viruses, toxins and infections; by eliminating pathogens and promoting tissue repair [2]. This process could be self-limiting (acute) or persistent and prolonged (chronic). Indeed, the chronic form is recognized as the most significant cause of death in the world today, with more than 50 % of all deaths being attributable to inflammation-related diseases such as ischemic heart disease, hypertension, diabetes mellitus, stroke, chronic kidney disease, non-alcoholic fatty liver disease, autoimmune and neurodegenerative conditions [3]. Studies have shown that identification and manipulation of pro-inflammatory cytokines may prevent or ameliorate the destructive effect on tissues and prevent progression to chronic diseases [4].

The African continent is endowed with abundance of medicinal plants. Most of these plants possess anti-inflammatory activity and hence, serve as first contact in meeting the primary health care needs of the people owing to the accessibility, affordability, cultural and spiritual acceptance, knowledge of its preparation and use. Presently, many drugs are being used as anti-inflammatories, worldwide. However, most of these drugs (e.g. non-steroidal anti -inflammatory drugs (NSAIDs) have been reported to have noxious side effects [5]. On the other hand, medicinal plants have been shown to have no or fewer side effects [6]. This has led to an intensive search for anti-inflammatory drugs with fewer side effects.

This search has focused on medicinal plants, which have been established by many studies to have anti-inflammatory properties [7]. This study therefore, investigates the in-vivo anti-inflammatory effect of the leaf extract of *B. pinnatum* on biomarkers of inflammation in Wistar albino rats. The effect of the extract on enzymatic indices will provide an insight into the likely mode of action of the plant.

Material

Reagents

All the chemicals and reagents used were of analytical grade standard obtained from Sigma-Aldrich, Inc (St Loius, MO) USA and water was glass distilled.

Equipment

GCMS (BUCK M910) equipped with a flame ionization detector, DR-200BC ELIZA Analyzer.

Methods

Preparation of plant material

Fresh green leaves of *Bryophyllum pinnatum* were collected from International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu-State. Identification and authentication of the plant was carried out at InterCEDD and a specimen was deposited at the center's herbarium. The plant material was shredded with a knife and air-dried under shade for 14 day.

Extraction of plant materials

The shade dried plant (leaves) was pulverized using a laboratory blender and the fine powder obtained stored in an air-tight container at room temperature until further use. The powdered sample was weighed and used for the extraction with 70 % ethanol (by maceration) for 72 hr; the yield of extracts was calculated using the formula below:

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Percentage yield = $\frac{Mass\ of\ extract after\ rotary\ evaporation\ (g)}{Mass\ of\ crude\ extract} \times 100$

Determination of Median Lethal Dose (LD_{50})

The median lethal dose was determined using Lorke's method (1983). This method has two phases which are phases 1 and 2 respectively.

Phase 1: This phase requires nine animals. The nine animals were divided into three groups of three animals each. Each group of animals was administered different doses (10, 100 and 1000 mg/kg) of the plant extract. The animals were placed under observation for 24 hours to monitor their behavior as well as their mortality.

Phase 2: This phase involves the use of three animals, which were distributed into three groups of one animal each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of the plant extract and then observed for 24 hours for behavior and mortality (Locke, 1983).

Then the LD50 was calculated with the formula:

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

D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality.

Determination of Phyto-chemical Components of the Extract Using GC-MS

Gas-chromatography analysis was performed using GC-MS SHIMADZU QP – 2010, JAPAN gas chromatography 5890 –11 with a fused GC column (0v -101) coated with polymethyl silicon (0.25 m x 50 m) and the conditions were as follows; temperature programming from 80°C-200°C held at 80°C for 1 minute at a rate of 5°C/min and at 200°C for 20 minute F/D temperature 300°C injection temperature 250°C, carrier gas nitrogen at a flow of 1 ml/min split ratio 1:75. The column length was 30 m with a diameter of 0.25 m and the flow rate of 50 ml/min the elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5 KV and sampling ratio of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank HERMLEZ 233 M-Z centrifuge Germany was used.

Identification of the Phytochemicals: The phytochemical components present in the extract their individual mass spectral peak value was compared with the database of National Institute of Science and Technology (NIST). The unknown chromatogram of the extract's phytochemicals was compared against a known chromatogram, peak value from the NIST Library database. Consequently, the details about their molecular formula, molecular weight, retention time and percentage content were obtained [8].

Experimental Animals

Wistar albino rats (30) weighing between 150 g-200 g were obtained from Chris Farm Ltd Mgbakwu, Awka, Anambra State. They were sorted, housedin standard cages with housing conditions of 12:12 light: dark cycles. They were fed with standard feed 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 (NIH; 85–23, 1985).

Grouping of Animals: The animals were grouped into 5 groups of 6 rats each as follows –

A – Untreated Control (2 ml distilled water)

B – Dexamethasone (Reference drug) – 25 mg/kg (extrapolated from adult dose)

C - *B. pinnatum* extract 100 mg/kg

D - *B. pinnatum* extract 200 mg/kg

E -*B. pinnatum* extract 400 mg/kg

The animals were administered the extract and drug for seven consecutive days with water and food ad libitum [9].

Dose Preparation: The hydro-ethanol extract of the dried powdered leaf of *B. pinnatum* was prepared in distilled water at three divided dose (100, 200, and 400) mg/kg, Dexamethasone (25 mg/kg) used as a reference drug, distilled water as untreated standard.

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 thegroin regionofeach anaesthetized rat.

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 5 ml 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.

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).

Results

The percentage yield of the extract is 23.75% (Table 1) (Figure 1 & Figure 2)

The effect of *B. pinnatum* extract on cyclooxygenase-1 activity was observed to be inhibitory in Groups B and C animals. The response of the animals to treatment was dose dependent (100, 200, and 400) mg/kg

Table 1. The list of phytochemicals identified with respect to the chromatogram obtained from GC – MS analysis of ethanol extract of the leave of *B. pinnatum.*

Peak	Compound	Molecular Formula	Molecular Weight (g/mol)	Retention Time (min)	% Content
1	Propanoic acid	CH_3CH_2COOH	74.08	5.9	13.502
2	2-Amino-3-hydroxypyridine	C5H6N_2O	110.11	6.757	34.678
3	(2,5-Dioxo-2,5-dihydropyrrole-1-yl)- acetic acid	$C_6H_5NO_4$	155.11	8.246	7.938
4	2-bromo-2-methyl ester	$C_5H_9BrO_2$	181.03	8.577	20.696
5	Pentanedioic acid	$C_5H_{10}O_2$	103.12	9.278	1.232
6	2-Cyclobutene-1-carboxamide 2-Thiophenecarboxylic acid	$C_5H_4O_2S$	128.15	10.054	7.422
7	Butanedioic acid	$C_4H_6O_4$	118.09	10.842	2.696
8	2-ethoxy-Thiazole	C_5H_7NOS	129.18	12.343	5.146
9	4-Chloro-2-nitrophenol	$C_6H_4ClNO_3$	173.55	18.43	6.692

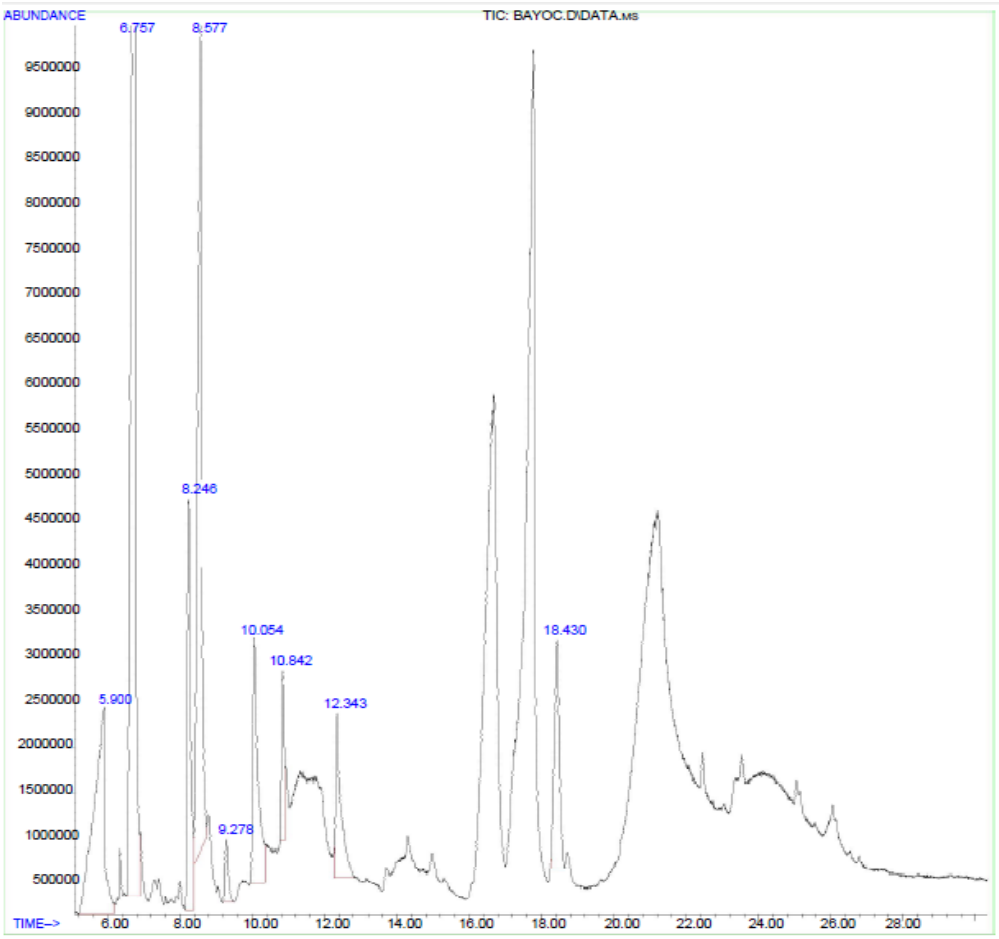

Figure 1. GC-MS Chromatogram of leaves of *B. pinnatum extract.*

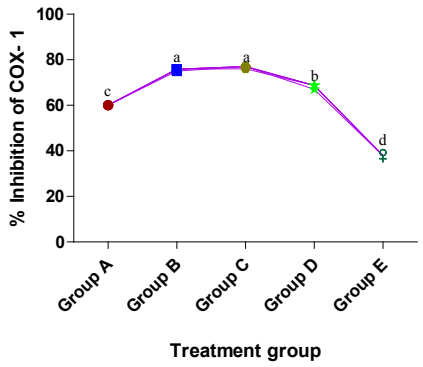

Figure 2. The effect of B. pinnatum leaf extract on Cycooxygenase-1 activity in experimental animals.

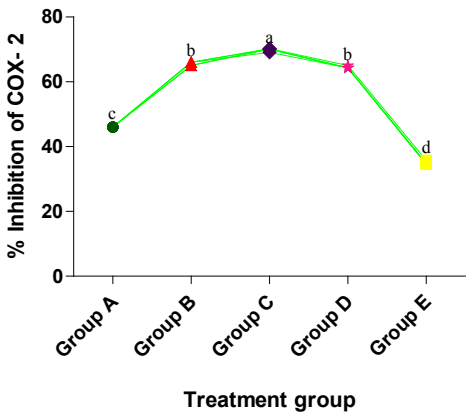

Figure 3. The effect of *B. pinnatum* leaf-extract on Cycooxygenase-2 activity in experimental animals.

body weight. A significant ($p \leq 0.05$) inhibition in the activity of COX-1 was observed. There was significant difference between the reference drug Dexamethasoneat 25 mg/kg dose and group E (distilled water -untreated control) (Figure 3).

The effect of the extract on COX-2 activity was significantly inhibited at 400 mg/kg dose (Group C). The activity was dose dependent. There was no significant difference between the 200 mg/kg of the extract and the 25 mg/kg of the reference drug– Dexamethasone. However, there was a significant difference between untreated control (distilled water treated group) and extract treated (100 and 200) mg/kg dose (Figure 4).

The response of the animals to treatment was dose dependent (100, 200 and 400) mg/kg body weight. There was significant inhibition of interlukin-1 activity in all experimental groups at $p \leq 0.05$ with the highest inhibition observed in group C animals (400 mg/kg dose) (Figure 5).

The response of the animals to treatment was dose dependent

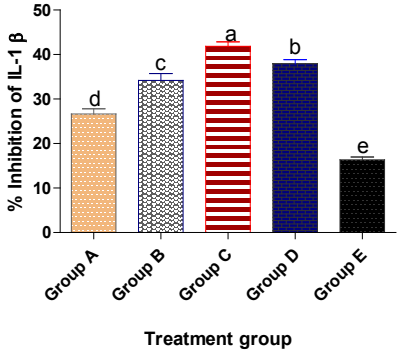

Figure 4. The effect of *B. pinnatum* extract on activity of Interleukin-1activity on experimental animals.

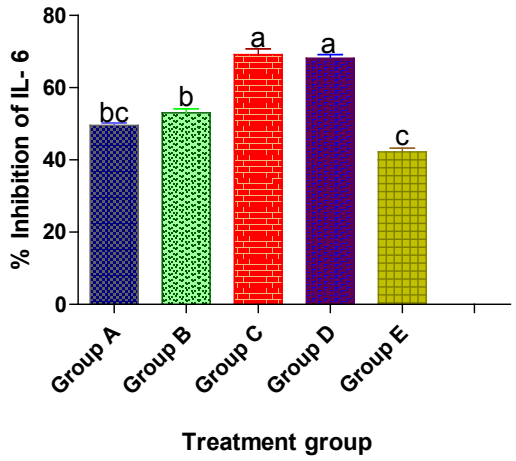

Figure 5. The effect of *B. pinnatum* leaf extract on interlukin-6 activity on experimental animals.

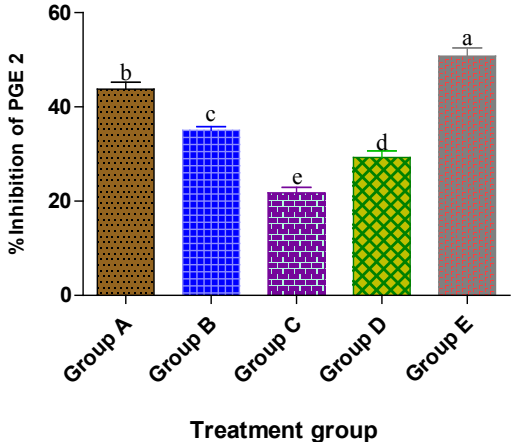

Figure 6. The effect of *B. pinnatum* leaf extract on Prostaglandin E2 on experimental animals.

(100, 200, 400) mg/kg body weight. There was significant inhibition of interlukins-6 in groups B and C animals. They showed significance when compared group E that was given distilled water (Figure 6).

The effect of the extract on Prostaglandin E2 in the experimental animals was dose dependent (100, 200, and 400) mg/kg body weight. PGE2 activity was significantly inhibited at 400 mg/kg dose (Group C).

Discussion

The percentage yield of *B. pinnatum* was appreciable - (23.75%). The extraction technique known as Green technique produced appreciable yield. Solvent extraction of plants is known to influence the yield of plants [10]. The GC-MS analysis of ethanol extract of the leaves of *B.pinnatum* showed nine peaks indicating the presence of nine different components in the extract as shown in Figure 1. The molecular formula, the molecular weight, the retention time and the percentage constituents of the compounds found in the extract are shown in Table 1.

The extract of leaves of *B. pinnatum* was found to be rich in 2-Amino-3-hydroxypyridine (34.678 % content). 2-Amino-3-hydroxypyridine is known to be useful in the preparation of clinical anti-inflammatory analgesics (Mudi and Ibrahim, 2008). It also form complexes with a number of transition metals, hence, inhibiting the corrosion of aluminium and copper in acidic solutions (Mudi and Ibrahim, 2008), and as well as undergoes condensation with 2-hydroxy-1-naphthaldehyde and 2-hydroxy-benzaldehyde to form Schiffs bases [11]. 2-bromo-2-methyl ester was also found to be present at relatively good quantity (20.696 % content) in *B. pinnatum* leaves (table 1.0). 2-bromo-2-methyl ester is an intermediate in the synthesis of hydroxamic acid (DIMBOA) which acts as an antifungal and anti-algal agent in crops [12]. It is also used in the synthesis of dextran macroinitiator for atom transfer radical polymerization (ATRP) by partial esterification of hydroxyl group of the polysaccharide [13].

Another compound found in appreciable amount in the leaf of the plant is propionic acid (13.502 % content). Propionic acid prevents the growth of fungus and molds, and its acid and salts can be used as a flavoring agent in some foods. Propionic acid has been used in bread and baked goods to inhibit mold growth, and it is also commonly used for the same purpose on the surface of cheeses (Edwards, 2003). This finding is supported by previous studies that plants contain many phytochemicals that possess various pharmacological activities [14].

The use of *B. pinnatum* for hypertension has generated a lot of interest in the plant. The in vivo effect of the extract on enzymatic indicators of inflammation is outlined in Figures 2-5.

Cyclooxygenase – 1 (COX-1)

The ethanol extract of *B. pinnatumshowed* an inhibitory effect on COX – 1 activity. This result is consistent with the findings of Caiazzo et al. (2016). This is an important observation as cyclooxygenase (COX) inhibitors are among the most commonly used drugs in the world for their anti-inflammatory and analgesic properties (Khan et al., 2019). COX-1is the enzyme that catalyzes the key step in the conversion of arachidonate PGH2, 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]. Thedecline 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 [19].

As depicted in Figure 3, that the inhibitory effect of the extract on cyclooxygenase 2 was increased ($p<0.05$) in extract treated animals. This result is consistent with the findings of Caiazzo [20]. COX-2 is the enzyme responsible for production of inflammatory mediators' prostagladin (PG) and its metabolites i.e. PGE2, PGF2, PGD2. It is produced by macrophages and is induced by TNF and EGF. COX-2 is also known as prostaglandin-endoperoxidase synthase and belongs to a family of isoenzymes responsible for the formation of prostanoid including thromboxane and prostaglandin such as prostacyclin from arachidonic acid. COX-2 is described to be able to modulate cell proliferation and apoptosis in solid tumor such as colorectal, breast and prostate cancer [21-23].

The inhibitory effect of the extract on Interleukin -1β (IL-1β) is shown in Figure 4 Interleukin (IL-1β) is an important mediator of inflammatory response and is involved in a variety of cellular activities including cell proliferation, differentiation, and apoptosis. They have been reported to be a potent inducer of COX-1 and TNFα and it produces highly effective antibodies but their pathophysiology is not yet well known [24].

Similarly, figure 5 presents the ability of the extract to inhibit Interleukin 6 (IL-6). Interleukin is involved in various physiological functions including neuro-development, hematopoiesis, bone metabolism and immunity it is implicated in many inflammatory diseases such as asthma, arthritis, osteoporosis, diabetes, and cancer [25,26]. IL-6 is a multi-functional cytokine that play roles in host defense and immune reactions [27]. Increased level of IL-6 has been reported in various pathological conditions such as infection (bacteria and virus), immune diseases, and inflammation (Narazaki and Kishimoto, 2018). Therefore, regulation of IL-6 might be effective against various diseases (Hunter and Jones, 2015).

Another distinct inflammatory biomarker that is of note is prostaglandin E2. Hui et al. (2010) reported similar result in atherogenic mice. PGE2 are

a group of physiologically active lipid compound called dinoprostone having diverse hormone-like effects in animals. It participates in a wide range of body functions such as contraction and relaxation of smooth muscle, dilation and constriction of blood vessels, control of blood pressure and modulation of inflammation [28,29]. 1001949 b t is found in almost every tissue in humans and other animals. They are derived enzymatically from the fatty acid - arachidonic acid. Prostaglandin E2 is released by blood vessel walls in response to infection or inflammation that act to induce fever [30].

Conclusion

The observations from the present study revealed that *B. pinnatum* leaf extract showed noteworthy inhibitions of clinically significant inflammatory biomarkers implicated in inflammatory pathways. It is of note that *B. pinnatum* leaf extract's effect on inflammatory biomarkers was significant and may be credited to the presence of pharmaceutical important bioactive components.

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