Original Research Article

Title: Blood cells formative properties of *B. pinnatum* in chronic inflammatory disorders: an experience with Wistar rats

ABSTRACT

The use of medicinal plants to resolve many ailments especially inflammatory diseases is gaining global attention. *Bryophyllum pinnatum*(Crassulaceae) called '*Oda-opue*' in Igbo, '*Eru-odundun* in Yoruba and '*Abomoda'* in Hausa languages is one of the plants widely used as food and medicines in tropical Africa, America, India and China. This study investigated the effect of ethanol leaf extract of *B. pinnatum* on haematological parameters in Wistar rats induced with chronic inflammation. 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 and a voucher specimen was deposited at the InterCEDD herbarium. The plant material was then shredded, air-dried under shade and pulverized. The fine powders obtained was weighed and extraction was done via solvent combination of water and ethanol (3:7) for 72 hr via maceration. The filtrate gotten was evaporated to dryness to obtain the ethanol extract which was used for further bioassay study. The phytochemical constituents of the plant extract were quantitatively determined by Gas Chromatography-Flame Ionization Detector (GC-FID). Chronic inflammation was induced intraperitoneally using cotton pellet and hematological parameters were analyzed using mindray hematology auto analyzer. Results showed that the leaf extract of *B. pinnatum* was rich in kaempferol (7.006 ±0.02 µg/g), sapogenin (3.372 ±0.02 µg/g), rutin (1.837 ±0.01 µg/g) and lunamarine (1.359 ±0.01 µg/g). Findings further showed a significant increase ($p<0.05$) in the total white blood cell (TWBC) as well as the red blood cell, haemoglobin and packed cell volume levels in the extract treated groups compared with the control group. The findings of this study showed the reversibility of the induced and suppressive effects of chronic inflammatory disorder on blood cell progenitors. It therefore becomes possible for the adjunct use of *B. pinnatum* in the management of anemia in chronic inflammatory disorders.

Keywords: Chronic disease, inflammation, health, medicinal plants.

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INTRODUCTION

The use of medicinal plants in the treatment of diseases has span through ages and currently gaining wide acceptance globally [1], as about 80 to 90 % of primary healthcare is sourced from traditional medicine worldwide [2]; hence, they remain a crucial part for drug development [3]. Several researchers have reported that plant metabolites are effective in the treatment of many ailments especially inflammatory diseases [4, 5]. The ease of availability, low cost, and least side

effects of plant based treatment therapies made it a focus of several available therapies most importantly in developing world [6].

Several medicinal plants have been reported to have diverse phytochemicals that can enhance erythropoiesis, protein synthesis and immune defense [7, 8], lower blood glucose, triglyceride and cholesterol levels [9]; possess anti-inflammatory, anti-oxidative, antimicrobial, renal and hepatic protective potentials [5, 10, 11].

Findings from traditional medical practitioners showed that *Bryophyllum pinnatum* is one of the promising plants useful in ameliorating several disease conditions especially chronic inflammatory diseases.

Bryophyllum pinnatum popularly called life plant belongs to the family of Crassulaceae and it is known locally as -*odundun* (Yoruba), *Odaa opue* or *Alupu* (Igbo) and *Abomoda* (Hausa) [1]. It is a crucial ethno-medicinal plant widely distributed in many parts of the world such as Europe, Madagascar, America, India, China, Asia and Africa [12]. It is a fast-growing, succulent perennial plant found in temperate, tropical and subtropical areas. It is also grown around houses and in gardens for both ornamental and medicinal purposes. The plant can grow to about 1.5 meter in height with leaves arranged in opposite direction [13]. Leaves have a wide spectrum of therapeutic potentials attributed to the rich phytochemicals such as flavonoids, triterpenes, alkaloids, steroids, saponins, glycosides, tannins, bufadienolides [5, 14]. Although *B. pinnatum* is used traditionally to treat so many illnesses including diabetes, liver and kidney diseases, dyslipidaemia, obesity, cough, wound, ulcer, infection and anaemia [15, 16], there is a dearth of literature reports of its importance on haematological profiles in a biological system. Hence, this study was carried out to evaluate the possible effects of the leaf extract of *B. pinnatum* on haematological parameters in Wistar rats induced with chronic inflammation.

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MATERIALS AND METHODS

Preparation of Plant Material

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 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.

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*., [17] using the formula below:

$$\text{Percentage yield} = \frac{\text{Mass of Extract after rotary evaporation (g)}}{\text{Mass of powdered sample (g)}} \times 100$$

Procurement of 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 Animal Research Ethics Committee of the Nnamdi Azikiwe University, Awka.

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* [18].

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 and was allowed to stay for seven days.

Collection of Blood Sample and Assay of Haematological parameters

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. Collected blood sample were analyzed at WeCare

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diagnostic center, Zik Avenue, Awka, Anambra State using mindray hematology auto analyzer (BC 5300).

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 $p<0.05$.

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RESULTS

The results of this study are presented in tables 1.0 to 3.0

The quantitative phytochemical content of the plant extract is presented in table 1.0. The plant extract contained high level of phenolic compound (9.22 µg) followed by Kaempferol (7.01 µg). Another compounds that were present in appreciable quantity include Sapogenin (3.37 µg), Rutin (1.83 µg) as well as Lunamarine (1.35 µg). Other constituents such as Spartein, Anthocyanin, oxalate, Ribalinidine, Phytate, Catechin and total flavonoids were present in trace quantity.

Table 1.0: Phytochemical constituents of ethanol leaf extract of *B. pinnatum*

Component	Concentration (µg/g)
Kaempferol	7.006 ±0.02
Spartein	0.005 ±0.00
Anthocyanin	0.097 ±0.01
Oxalate	0.196 ±0.03
Sapogenin	3.372 ±0.00
Rutin	1.837 ±0.00

Lunamarine	1.359 ±0.01
Ribalinidine	0.027 ±0.00
Phytate	0.234 ±0.03
Catechin	0.235 ±0.03
Total flavonoid	0.276 ±0.02

Values are means ± standard error of mean.

The effect of *B. pinnatum* leaf extract on hematological parameters (Total white blood cell, neutrophil, lymphocyte, monocyte, eosinophil, basophil and platelet) is presented in table 2.0. There was a significant increase ($p<0.05$) in the TWBC levels of group C and D animals compared with the control group. Group D animals showed significant increase in the neutrophil content compared with the control group. The lymphocyte level of group C animals was found to be highest and significantly different from the control group.

Table 2.0: Effect of *B. pinnatum* leaf extract on Hematological parameters

Treatment group	TWBC (×10ˆ9/L)	Neut. (×10ˆ9/L)	Lymp. (×10ˆ9/L)	Mon. (×10ˆ9/L)	Eos. (×10ˆ9/L)	Bas. (×10ˆ9/L)	PLT (×10ˆ9/L)
Group A	7.95 ± 0.28^{c}	4.35 ± 0.53^{c}	3.80 ± 0.42^{c}	0.24 ± 0.14^{a}	0.04 ± 0.01^{c}	0.03 ± 0.01^{b}	640.33 ± 1.53^{c}
Group B	8.60 ± 1.15^{b}	4.39 ± 0.18^{d}	4.00 ± 0.59^{b}	0.29 ± 0.02^{c}	0.06 ± 0.02^{c}	0.04 ± 0.05^{a}	646.67 ± 2.89^{a}
Group C	10.35 ± 1.41^{a}	4.55 ± 0.22^{d}	6.01 ± 1.30^{a}	0.31 ± 0.06^{b}	0.14 ± 0.07^{b}	0.07 ± 0.05^{a}	682.67 ± 2.08^{b}
Group D	10.49 ± 1.28^{a}	5.59 ± 2.08^{a}	5.31 ± 0.53^{b}	0.33 ± 0.13^{a}	0.39 ± 0.19^{a}	0.07 ± 0.02^{b}	782.67 ± 1.15^{d}
Group E	7.63 ± 0.28^{c}	3.78 ± 0.77^{b}	2.57 ± 0.40^{c}	0.20 ± 0.13^{a}	0.02 ± 0.01^{c}	0.01 ± 0.01^{b}	579.00 ± 2.65^{b}

Values are means ± standard error of mean. Values on the same column with different alphabet superscript are significantly different at P< 0.05.

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TWBC: Total white blood cell; **Neut.:** Neutrophil; **Lymp.:** Lymphocyte; **Mon.:** Monocyte; **Eos.:** Eosinophil; **Bas.:** Basophil; **PLT.:** Platelet.

The effect of *B. pinnatum* leaf extract on RBC, HGB, PCV, MCV, MCH and MCHC is presented in table 3.0. There was a significant increase ($p<0.05$) in the RBC,HGB, PCV, MCV, MCH and MCHC levels of extract treated groups compared with the control group.

Table 3.0: Effect of *B. pinnatum* leaf extract on RBC, Hgb, PCV, MCV, MCH and MCHC

Treatment group	RBC (×10ˆ12/L)	HGB (g/L)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/L)
Group A	6.97± 0.01^{d}	131.33± 2.08^{b}	4.23± 0.15^{a}	49.04±0.04^{b}	20.02±0.14^{b}	315.12±0.06^{c}
Group B	7.41± 0.04^{d}	134.00± 2.00^{c}	4.33± 0.04^{d}	54.22±0.12^{a}	21.12±0.18^{a}	318.02±0.09^{b}
Group C	7.70± 0.13^{c}	142.30± 2.52^{a}	4.69± 0.10^{b}	56.28±0.02^{b}	24.22±0.06^{c}	320.16±0.18^{a}
Group D	7.47± 0.62^{a}	143.67± 1.15^{d}	4.93± 0.08^{c}	59.03±0.14^{a}	25.02±0.06^{c}	328.05±0.10^{b}
Group E	6.21± 0.24^{b}	119.33± 2.08^{b}	3.40± 0.07^{c}	48.00±0.00^{c}	18.23±0.04^{d}	316.00±0.04^{c}

Values are means ± standard error of mean. Values on the same column with different alphabet superscript are significantly different at P< 0.05.

RBC: Red blood cell; **HGB:** Heamoglobin; **PCV:** Packed cell volume; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration.

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DISCUSSION

Plants play important roles in discovery 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 ±0.02 µg/g) (table 1.0). This is consistent with the report of Barve *et al*. [19]. Kaempferol is a natural flavonol, yellow chrystalline solid, and highly soluble in ethanol. It is known to reduce the risk of chronic diseases, especially cancer [20]. It has been shown to augment human body's antioxidant defense against free radicals [21], and modulates apoptosis, angiogenesis, inflammation and metastasis [22].

The sapogenin content (3.372 ± 0.00µg/g) (table 1.0) 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 [23]. 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 [24].

Rutin is another compound that is present in an appreciable quantity (1.837± 0.01 µg/g) (Table 1.0). Rutin is a bioflavonoid with powerful antioxidant properties [25]. Rutin has been shown to

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reduce body weight by 7.9 % [26], improve eye health by strengthening fragile capillaries [27], and as well posses anti-inflammatory effects [28].

Other compounds such as spartein (0.005±0.00 µg/g), ribalinidine (0.027±0.00µg/g) and anthocyanin (0.097±0.01 µg/g) (Table 1.0) were present in minute quantities.

The assessments of hematological parameters are useful guide to ascertaining the effect of foreign substances including plant extracts in a biological system. They are used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histopathology of the organs [29, 30].

Tables 2.0 and 3.0 showed the effect of *B. pinnatum* leaf extract on Hematological parameters. As evident in table 2.0, there was significant increase ($p< 0.05$) in a dose dependent manner in the total white blood cell (TWBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil and platelet of the treated groups when compared with the control group. This result is in agreement with the findings of Ahumibe and Braide [31] and also supports the observation made by Nwankpa *et al*. [32] who suggested that increase in white blood cells helps to stimulate cytokine erythropoietin. *B. pinnatum* extract therefore, might have plausibly stimulated cytokine erythropoietin which consequently may have stimulated blood cell synthesis.

White blood cell (WBC) and its differentials (lymphocytes and neutrophils) and other haematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function [33]. WBC's are essential for the protection of the animal against foreign invaders [8]. Elevation in their levels is indicative of response to an immunological challenge. Neutrophil are important phagocytic cells normally elevated in the early inflammatory response [34], while lymphocytes are subtypes of leucocytes critically essential for providing cell mediated immunity. In addition, increase in WBC and neutrophil counts suggest the ability of the

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leaf extract of *B. pinnatum* to boost the cells immune system since they function as active phagocytic agents against foreign compounds [35]. This may possibly explain its use in the management of inflammation and other related ailments.

Similarly, a significant increase ($p<0.05$) in RBC, Hgb, PCV, MCV, MCH and MCHC were observed in the treated groups compared to the control (table 2.0). The result of this study is similar to the report of Esenowo *et al*. [36] and Okon *et al*. [37] in their separate studies. Although the result on RBC, Hgb, and PCV in this study is in agreement with the findings of Ogbonnia *et al* [38] and Nwankpa *et al*. [32], the result of MCV concentration which showed a slight increase on administration of leaf extract of *B. pinnatum* is in contrast with their result.

This hematopoietic condition may be due to different mechanisms which include increase in rate of blood cell synthesis and or decrease in rate of blood cells destruction. Any of the two mechanisms may have been responsible for the increase in the red cell indices. The plant extract may have the potential to stimulate erythropoietin release which consequently increases the synthesis of red cells [39] and or help fight against infections and microbial invasions [37] which destroys blood cells both of which may culminate to increases in blood cells as observed in this study.

CONCLUSION

The observations from the present study revealed similarity in the phytochemistry of the plant compared to the reports from the literature. The result on haematological parameters demonstrated that the extract is capable of stimulating blood cell formation and act as active phagocytic agent against foreign compounds. This could be attributed to many bioactive compounds present in the extract which could be synthesized to produce new plant based product to fight inflammatory disorders with fewer side effects.

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