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Antifungal and Immunomodulatory Activity of *Bryophyllum pinnatum* Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Author JEO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors LE and FIE managed the analyses and literature searches of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to investigate the antifungal and immunomodulatory activities of *Bryophylum pinnatum*. Both aqueous and ethanol solvents were used for extraction. Five fungal species including *Aspergillus niger, Aspergillus flavus, Cladosporium herbarium, Candida albicans* and *Penicillium italicum* were obtained from the University of Benin Teaching Hospital and they were preliminarily identified using standard microbiological methods. Wistar rat models for the study were purchased and acclimatized for a period of two weeks. The rats were divided into five groups and orally administered with the ethanol extract of the plant while one group served as control. Antimicrobial and hematological parameters including packed cell volume (PCV), hemoglobin, white blood cell counts, platelets and CD_4 count were assayed using standard methods. The only fungus sensitive to aqueous extract was *Aspergillus niger*, with zone of inhibition ranging from 6.00±0.58-11.33±0.33 mm at concentration range of 50-100 mg/ml. Mean zones of inhibition of ethanolic extract ranged from 11.67±0.67-20.33±0.33 mm against

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Cladosporium herbarium at concentration range of 6.25-100 mg/ml. Minimum inhibitory concentrations (MIC) of ethanol extract ranged from 6.25- 50 mg/ml against fungal isolates. While MIC of aqueous extract was 50 mg/ml against susceptible fungal isolate. Minimum fungicidal concentrations of ethanol and aqueous were 25- 100 mg/ml and 100 mg/ml respectively. Significant difference was observed between the treatment and control groups in platelet counts (range: 95.40±1.86-126.20±5.40% and control: 108.60±4.19%), PCV (range: 39.00±0.71-44.20±0.58%; control: 39.00±0.71%) and hemoglobin (range:12.94±0.21-14.62±0.24 g/dl; control: 12.94±0.21 g/dl). There was no significant difference between the treatment and control groups in CD_4 counts (75.40±19.32-99.00±6.33cells/ml and control 75.4±19.32 cells/ml). *Bryophylum pinnatum* has been shown in this work to possess both antimicrobial and hematological properties.

Keywords: Fungicidal; immunomodulatory; phytochemical; extract, inhibition.

1. INTRODUCTION

Bryophyllum pinnatum (*Kalanchoe pinnata*; Lamarch Crassulaceae) is an erect, succulent, perennial shrub that grows about 1.5m tall and reproduces through seeds and also vegetatively from leaf bubils. It has a tall hollow stem, freshly dark green leaves that are distinctively scalloped and trimmed in red and dark bell-like pendulous flowers [1]. *Bryophyllum pinnatum* can easily be propagated through stems or leaf cutting. It is an introduced ornamental plant that is now growing as a weed around plantation crop and widely used in traditional medicines [2]. Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases and heal injuries. Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial and immunomodulatory functions [3]. The secondary metabolites of plants provides humans with numerous biological active products which have been used extensively as drugs, foods, additives, flavours, insecticides, colorants, fragrances and chemicals. These secondary metabolites include several classes such as saponins, terpenoids, phenolic compounds, steroids, alkaloids and flavonoids [4]. In recent years, researches into new sources of chemotherapeutic agents have intensified due to failure or non-effectiveness of conventional drugs to which many pathogens have developed resistance. Many compounds with potential biological activities have been isolated from many plants. In this regard, *Bryophyllum pinnatum* is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhoea and cithiasis [5]. In South eastern Nigeria, this herb is used to facilitate the dropping of the placenta of new born baby [6]. The lightly roasted leaves are used externally for skin fungus and inflammations. The leaf infusions are an internal remedy for fever [4]. Different naturally occurring flavonoids have been described and subcategorized into flavones, falvans, flavanones, isoflavonoids, chalcones, aurones and anthocyanidines. These flavonoids exhibit remarkable biological activities including inhibitory effects on enzymes, modulatory effects on some cell types, protection against allergies, antiviral, anti-malarial, antioxidant, anti-inflammatory and anti-carcinogenic properties [7].

Phytochemical screenings of *Bryophyllum pinnatum* have yielded Alkaloids, Triterpenes, Glycosides, Flavonoids, Steroids, Butadienolides, Lipids, and organic acids, Phenol and Tannis, free amino acid and Terpenoids. Arachidic acid, Astragalin, Behenic acid, beta Amyrin, Benzenoids, Bersaldegenin, beta-Sitosterol, Bryophollenone, Bryophollone, Bryophyllin, Caffeic acid, Ferulic acid, Quercetin, Steroids, Taraxerol have also been found from extracts of *Brophyllum pinnatum* [8].

Two novel Flavanoids; 5 Methyl 4,5,7 trihydroxyl flavones and 4,3,5,7 Tetrahydroxy 5 methyl 5 propenamine anthocyanidines from this plant have shown potential antimicrobial activities against *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *E. coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. When 60% methanolic extract of *Bryophyllum pinnatum* leaf was used to inhibit the growth of bacteria, at a concentration of 25 mg/ml it showed good antibacterial effects. Further the Plant is effective in the treatment of typhoid fever and other bacterial infections, particularly those caused *by S. aureus, E. coli, B. subtilis, P. aeruginosa, K. aerogenes, K. pneumonia and S. typhi* due to the presence of phenolic compounds [9]. The anti-inflammatory potential of *Bryophyllum pinnatum*

was investigated by [10]. However, the antifungal and immunomodulating activity of the plant has not been well studied. Therefore, this work was carried out to determine the immunomodulatory properties and antifungal activities of *Bryophyllum pinnatum* leaf extract.

2. MATERIALS AND METHODS

2.1 Plant Material

Bryophylum pinnatum leaves were obtained from Adolor Street in Benin City and identified at the Herbarium, Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. The leaves were air-dried, ground using sterilized laboratory blender. The powdered leaf was kept in a sterile bottle container until required.

2.2 Preparation of Crude Extracts

Fifty grams (50 g) of the grinded *Bryophylum pinnatum* leaves was soaked in 250 ml each of distilled water and ethanol for 24 hrs. The extract was filtered through a sieve with pore size of 250µm to remove debris. The filtrate was then filtered through membrane filter paper. The final filtrate was evaporated in a water bath at 40°C to get the crude extract. The crude aqueous and ethanol extracts were stored at 4°C until required [11].

2.3 Preparation of Concentration of Plant Extract

One gram (1g) each of both ethanol and aqueous extract was added to 10ml of ethanol and distilled water respectively to give a concentration of 100mg/ml. Other concentrations of 50, 25 and 12.5 and 6.25mg/ml were prepared by double dilution method. In this procedure, 1ml of content in the stock concentration (containing 100mg/ml) was added to 1ml each of distilled water and ethanol in test tubes to give 50mg/ml concentration. 1ml of this 50mg/ml is added to another test tube of 1ml distilled water and ethanol to give 25mg/ml and so on [12].

2.4 Test Microorganisms

Five fungal isolates, *Aspergillus niger, Aspergillus flavus, Cladosporium herbarium, Candida albicans* and *Penicillium italicum* were used in this study. The fungi were obtained from the Microbiology Laboratory stocks in University of Benin Teaching Hospital. They were then identified based on their cultural and microscopic characteristic before further work on them were carried out.

2.5 Fungal Inoculum Preparation

The fungi inocula were prepared by inoculating the test organisms on potato dextrose broth and kept at room temperature for 24hr. 0.2 ml of the different fungal broth culture was used for the antifungal study [12].

2.6 Agar Well Diffusion Technique

The ability of the various extracts to inhibit the growth of the clinical test organisms was determined using the agar well technique. The inoculated potato dextrose agar plates were allowed to dry. After which, wells were bored on the surface of inoculated agar plates using 4mm cork borer. Zero point one millilitres (0.1ml) of the different concentration of each extracts was transferred into the well using Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were kept at room temperature for 24hr. The experiment was performed in triplicate and the resulting zones of inhibition were recorded as mean ± standard error [13].

2.7 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The minimum inhibitory concentration (MIC) of the extracts was determined for each of the test organisms at varying concentrations of 100, 50, 25, 12.5 and 6.25mg/ml. 1 ml of potato dextrose broth and 1 ml each of the extracts were added to test tubes and a loopful of fungi isolates was introduced. A tube containing Potato dextrose broth only was seeded with the fungi isolates to serve as control and all tubes were kept at room temperature for 24hr to check for growth. The minimum fungicidal concentration of the plant extract was carried out according to [14]. Briefly, 1 ml fungal culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and subcultured on to potato dextrose agar. The agar plates were left at room temperature. After incubation the concentration at which there was no single growth of fungi was taken as MFC.

2.8 Experimental Animals

Eight weeks old Wistar rats weighing between 200-270 g were used in this experiment. The rats were housed at the Department of Microbiology Animal House and were fed with pelleted rat food and water daily. After acclimatization for two weeks, rats were divided into six groups, with five rats in each group. Temperature ranged from 30°C to 37°C during the period of experiment. The groups were labelled A to F. Group F served as the control group and did not receive administration of extract. Groups A to E received different dosage of the ethanolic plant extract. Group A received 300mg/kg, group B-250mg/kg, group C – 200mg/kg, group D – 150mg/kg and group E -100mg/kg body weight. On Day 15, 2.5ml of blood sample was collected from each rat into EDTA anticoagulant container and was used to estimate the counts of CD_4, neutrophils, lymphocytes, eosinophils and total leukocytes of each group [15].

2.9 FULL Blood Count (FBC)

This was carried out using Symex21N Japan model auto-analyzer. Automated cell counters was used to quantify and classify the different cell populations according to [16].

2.10 Enumeration of CD_4Count

This was carried out using Partec CyFlow Counter (CY-S-3022 model). Briefly, 20µl whole blood was added to a Partec test tube and 20µl of CD4 mAb PE was added. This was gently mixed and incubated for 15min at room temperature in the dark. After incubation, 800µl of no lyse buffer was added and shaken gently. The sample was then analyzed on the Partec device [16].

2.11Statistical Analysis

Data were analysed using statistical package for social sciences (SPSS) version 23.0. Descriptive statistic was used to present values in means and standard errors. One way ANOVA was used to find significant difference among different parameters [17].

3. RESULTS

The zone of inhibition of aqueous extract of *Brophyllum pinnatum* against funga isolates is shown in Table 1. Many fungi species were resistant to all concentrations of aqueous extract of *B*. *pinnatum* except the fungus *Aspergillus niger* with zones of inhibition ranging from 6.0±0.58 – 11.33±0.33 mm at concentration range of 50-100mg/ml.

A comparatively higher antifungal activity for ethanolic extract was observed with *Cladosporium herbarium*, *Candida albicans* and *Aspergillus niger* as shown in Table 2. The order of antifungal activity was *Aspergillus niger* (50-100mg/ml) < *Candida albicans* (25-100mg/ml) < *Cladosporium herbarium* (6.25-100mg/ml).

Minimum antifungal activity of aqueous extract was also observed against fungal isolates when compared with ethanolic extract and higher zones of inhibition were observed at higher concentrations in susceptible fungi species.

The minimum inhibitory concentration of ethanolic extract against fungi species as shown in Table 3 ranged from 6.25-50mg/ml while that of aqueous extract was 50mg/ml. The minimum fungicidal concentration of ethanolic extract ranged from 25-50mg/ml while that of aqueous extract was 100mg/ml against susceptible fungal isolates.

Effects of ethanolic extract of *B*. *pinnatum* on haematological parameters of wistar rat models is shown in Table 4. The PCV values ranged from 39.00±0.71- 44.20±0.58% with the control being 39.00±0.71%. Haemoglobin values ranged from 12.94±0.21-14.62±0.24g/dl, while the control was 12.94±0.21g/dl.

Effect of ethanolic extract of *B*. *pinnatum* on CD4 count as shown in Table 5 has values ranging from 81.60±2.84- 99.00±6.33 cells/ml while the control was 75.40±19.32 cells/ml. There was no significant difference between treated and control groups.

4. DISCUSSION

Plants have been reported to be vast repertoire of bioactive phytochemical compound. These compounds which include flavonoids, alkaloids, tannins etc., are usually responsible for the various biologic properties of the plant, including antimicrobial and other medicinal properties. It has been reported that organic solvent such as ethanol, will usually extract more of the bioactive phytochemical component of the plant compared to aqueous solvent, hence the reason for higher antibacterial activity in the ethanolic fraction of the leaf extract [18].

Table 1. Zone of inhibition of aqueous extract of *Bryophyllum pinnatum* (mm) against fungal isolates

Test organism	Concentrations (mg/ml)				
	100	50	25	12.5	6.25
Penicillium italicum	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Cladosporium herbarium	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Candida albicans	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Aspergillus flavus	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Aspergillus niger	11.33±0.33	6.0±0.58	0.0±0.0	0.0±0.0	0.0±0.0

Table 2. Zone of inhibition of ethanolic extract of *Bryophyllum pinnatum* (mm) against fungal isolates

Test organisms	Concentration (mg/ml)				
	100	50	25	12.5	6.25
Penicillium italicum	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Cladosporium herbarium	20.33±0.33	15.67±0.67	12.33±0.33	12.0±0.58	11.67±0.67
Candida albicans	19.0±0.58	18.0±0.58	14.67±0.33	0.0±0.0	0.0±0.0
Aspergillus flavus	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Aspergillus niger	14.0±0.58	9.33±0.33	0.0±0.0	0.0±0.0	0.0±0.0

Table 3. Minimum inhibitory concentration and minimum fungicidal concentrations of ethanolic and aqueous extracts of *B. pinnatum*

Test organisms	MIC(mg/ml)		MFC (mg/ml)	
	Ethanol	Aqueous	Ethanol	Aqueous
Penicillium italicum	ND	ND	ND	ND
Cladosporium herbarium	6.25	ND	25	ND
Candida albicans	25	ND	50	ND
Aspergillus flavus	ND	ND	ND	ND
Aspergillus niger	50	50	100	100

ND- Not determined

Table 4. Effect of ethanol extract of *B. pinnatum* on haematological parameters of rat

Groups	Parameters	
	PCV(%)	Hb(g/dl)
A	^{b}42.6 ± 0.93	^{b}14.16±0.31
B	^{b}42.2±1.16	^{b}14.0±0.39
C	^{b}43.0±1.70	^{b}14.3±0.53
D	^{b}44.2±0.58	^{b}14.62±0.24
E	^{b}42.8± 0.86	^{b}142.24± 0.29
F	^{a}39.0± 0.71	^{a}12.94±0.21

Letters A-E stands for the groups of organism administered 300, 250, 200, 150 and 100 mg/kg of the ethanolic extract respectively while group F is the control. Groups with similar superscript as control has no significance while groups with different superscript describes the significance at P <0.05

Table 5. Immuno-modulatory effects of ethanol extract of *B. pinnatum* on white blood cells proliferation

Parameters	Groups					
	A	B	C	D	E	F
WBC(×10^3)	6.04±0.12^{b}	5.16±0.25^{a}	5.52±0.24^{a}	4.90±0.25^{a}	4.88±0.21^{a}	4.96±0.10^{a}
Neut(%)	44.8±3.69^{a}	36.2±1.53^{a}	36.4±9.18^{a}	43.4±1.86^{a}	33.3±1.48^{a}	39.2±4.40^{a}
Lymp(%)	49.2±3.15^{a}	59.2±1.24^{a}	57.4±10.78^{a}	52.2±1.88^{a}	62.8±2.67^{a}	53.2±3.02^{a}
Platelet(×10^3)	105.8±4.10^{a}	105.8±4.60^{a}	126.2±5.40^{c}	103.0±3.38^{a}	95.4±1.86^{a}	108.6±4.19^{b}
CD4(cells/ml)	81.6±2.84^{a}	95.8±2.08^{a}	99.0±6.33^{a}	86.2±4.59^{a}	91.6±4.07^{a}	75.4±19.32^{a}

Letters A-E stands for the groups of organism administered 300, 250, 200, 150 and 100 mg/kg of the ethanolic extract respectively while group F is the control. Groups with similar superscript as control has no significance while groups with different superscript describes the significance at P <0.05

Many of the fungi species were resistant to the all concentrations of the aqueous plant extract. The only sensitive fungus was *Aspergillus niger*. This resistance may have resulted from the inability of the plant extract to penetrate the cell wall of the fungal isolates. Comparatively higher antifungal activities were observed in the ethanol extract. Only *Penicillium italicum* and *Aspergillus flavus* were completely resistant to all concentrations of the plant extract. Minimum inhibitory concentrations of ethanol extract was lower than that of the aqueous extract indicating a higher efficacy of the ethanol extract. Similarly, Minimum fungicidal concentrations of ethanol extract was also lower than that of aqueous extract. Zakharchenko et al. [19] isolated the antimicrobial peptide cecropin P1 from *Bryophyllum pinnatum* and reported its fungicidal activity against dermatomycoses and even in treatment of wound infected with fungi. In their work, the antifungal activity observed against *Candida albicans* agrees with findings from this work as the ethanol extract of the plant was active against *Candida albicans* in this work. However, there was no in vivo study in this work compared to their work.

There were changes in haematology parameters in experimental animals administered with ethanol extract of *Bryophyllum pinnatum,* compared to the control group. Observable changes between experimental and control animals are indication that ethanol extract of the plant has modulatory propensity on haematological parameters. Ethanolic extract of *Brophyllum pinnatum* has been shown in this work to increase the PCV and haemoglobin levels. No significant variation was observed between white blood cells proliferation of experimental animals and control group. Increase in haemoglobin enhances the oxygen transporting properties of the red blood cells as a result of increased number of red cell. The observed increase in the PCV recorded in this study on administration of extract of *Bryophyllum pinnatum* may have been due to effect on bone marrow stem cell by improving its proliferative activity [20]. There was a significant difference in platelet numbers between treated and control groups. The reduction in platelet counts suggests that continuous intake of this extract needs to be checked. There was no significant difference in white blood cell (WBC) parameters between treated and control group except for group A that received highest concentration of the extract (300mg/kg body weight). This means that the ethanolic extract of *B. pinnatum* may increase the WBC at higher concentrations. No significant difference was observed in the CD4 counts of treated and control groups. This could also be due to the concentration of the extract used as higher concentration may register significant increase. Results showed that ethanolic extract of leaf caused increased haematological parameters that help in primary and secondary clearance of invading pathogens. This is in line with the work of [18] who reported that crude methanolic leaf extract of *B. pinnatum* has properties that increase the haemoglobin, packed cell volume and total white blood cells while decreasing the platelets. However, in contrast to their findings, the amount of platelet was found to increase at extract concentration 200mg/kg of experimental animals. In a research findings by [21], aqueous extract of *Bryophyllum pinnatum* elevated white blood cell count, reduced neutrophil count without affecting lymphocyte count and packed cell volume, when compared to control, thus agreeing with the findings from this study that the extract possesses immunomodulating potentials.

The phytochemical constituents of the leaf extract of *B. pinnatum* may have stimulatory effect on the bone marrow for leucocyte proliferation and haemoglobin production. This may be as a result of tannin, ascorbic acid [6] and phenolic content. Other phytochemicals that may have affected the haematological parameters in this study include flavonoids, zinc, riboflavin and niacin [22].

5. CONCLUSION

This work has shown that *Bryophyllum pinnatum* ethanol and aqueous extracts have moderate antifungal activity. However, the ethanol extract was more active compared to the aqueous extract. Furthermore, moderate immune-modulatory activity was observed in Wistar rat models with an increase in platelet count, haemoglobin and PCV. *B. pinnatum* has been shown to possess hematological properties in this study.

ETHICAL APPROVAL

Ethical approval for the experimental protocol was obtained from the University of Benin Ethics Committee and care of animals was taken as per guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) (with reference number: FLS/17/101).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jaiswal S and Sawhney S. Correlation of epiphyllous bud differentiation with foliar senescence in crassulacean succulent *Kalanchoe pinnata* as revealed by thidiazuron and ethrel application. Journal of Plant Physiology. 2006;163:7177-722
2. Nagaratna A, Prakash L and Hegde A. comprehensive review on Parnabeeja (*Bryophyllum pinnatum* (lam.). Journal of Medicinal Plants Studies. 2015;3(5):166-171.
3. Gill LS. Ethno-medical uses of plant in Nigeria: UNIBEN Press. 1992;46.
4. Okwu DE and Nnamdi FU. Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. Pharmceutical Chemistry Journal. 2011;3(2):1-10.
5. Mahendra Y, Vijay DG, Manish MW. *Bryophyllum pinnatum* leaf extracts prevent formation of renal calculi in lithiatic rats. Ancient Science Life. 2016;36(2):90-97.
6. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicnal plants. African Journal of Biotecnology. 2006;5(4):357-361.
7. Ozolua RI, Eboka CJ, Duru CN and Uwaya DO. Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. Nigerian Journal of Physiology and Science. 2010;25:149-157
8. Joshi A, Chauhan RS. Phytochemical analysis and Cytotoxicity studies of *Bryophyllum calycium*in BHK-21 cells. Scholars Academic Journal of Pharmacy. 2013;2(3):190-194.
9. Afzal M, kazmi I, Singh R, Chauhan M, Bisht T and Anwar F. *Bryophyllum pinnatum*: A review. International Journal of Research in Biological Sciences. 2012;2(4):143-149.
10. Ojewole JAO. Antinociceptive, anti-inflammatory and anti-diabetic effects of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract. Journal of Ethano-pharmacology. 2005;99:13-19
11. Abdulazeez AA, Hassan AO, Adewole AA, Fadairo JK. Synergistic effect of combined extract of *Bryophyllum pinnatum* and *Aloe barbadensis* enhances antimicrobial activity *in-vitro*. Global Advanced Research Journal of Medicine and Medical Science. 2014;3(1):26-32.
12. Aneja KR. Experiments in microbiology, plant pathology and biotechnology (4th ed.). New age international publishers, New Delhi, Bangalore. 2003;607.
13. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases. 2009;49: 1749-1755
14. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in Southwest Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complementary and Alternative Medicine. 2005;5-6.
15. Ilevbare L, Evbuomwan L. Hematological and immunomodulatory activity of *Ocimum basilicum*. International Journal of Novel Research in Life Sciences. 2018;5(3):19-25.
16. Lewis SM, Bain BJ, Bates I. Basic haematological techniques In: Practical haematology. Lewis, S. M., Bain, B. J. and bates, I. (editors). Churchill Living stone Elsevier, Philadephia, Pennsylvania. 2006;26-57.
17. Ogbeibu AE. Biostatistics: A practical approach to research and data handling. Mindex Publishing Company, Benin City, Nigeria. 2015;264.
18. Ufelle SA, Ukaejiofo EO, Neboh EE, Achukwu PU, Ghasi S, Ikekpeazu EJ, Maduka IC. The effect of crude methanolic leaf extract of *Bryophyllum pinnatum* on some haematological parameters in Wistar rats. Asian Journal of Medical Sciences. 2011;3(3):121-124.
19. Zakharchenko NS, Belous, AS, Biryukova YK, Medvedeva OA, Belyakova AV, Masgutova GA, Trubnikova EV, Buryanov YI and Lebedeva1 AA. 2017. Immunomodulating and Revascularizing Activity of Kalanchoe pinnata Synergize with Fungicide Activity of Biogenic Peptide Cecropin P1. Journal of Immunology Research Article ID 3940743. 2017;1-9.
20. Fakeye TO, Anirban P, Bawankule DU, Khanuja SPS. Immunomodulatory effect of extracts of *Hibiscus sabdariffa* L. (Family Malvaceae) in a mouse model. Phytother Res. 2008;22:664–668.

21. Aprioku JS, Igbe I. Effects of Aqueous *Bryophyllum pinnatum* Leaf Extract on Haematological, Renal and Sperm Indices in Wistar Rats. Indian Journal Pharmaceutical Science. 2017;79(4):521-526

22. Salahdeen HM, Yemitan OK. Neuropharmacological effects of aqueous leafextract of *Bryophyllum pinnatum* in mice. African Journal of Biomedical Research. 2006;9:101-107.

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