Istanbul J Pharm 52 (3): 271-280
DOI: 10.26650/IstanbulJPharm.2022.1007696
Original Article

Protective effects of *Brenania brieyi* (De Wild) E.M.A.Petit root bark fractions against inflammatory-mediated hemolysis and dyslipidemia in rats

Ifeoma Felicia Chukwuma[1], Victor Onukwube Apeh[2], Florence Nkechi Nworah[1], Felix Ifeanyi Nwafor[3], Lawrence Uchenna Sunday Ezeanyika[1], Victor Nwadiogo Ogugua[1]

[1]University of Nigeria, Nsukka, Department of Biochemistry, Nigeria
[2]Federal College of Dental Technology and Therapy, Enugu, Department of Applied Sciences, Nigeria
[3]University of Nigeria, Nsukka, Department of Pharmacognosy and Environmental Medicine, Nigeria

ORCID IDs of the authors: I.F.C. 0000-0001-9629-213; V.O.A 0000-0003-2987-4046; F.N.N. 0000-0002-7724-9846; F.I.N. 0000-0003-1889-6311; L.U.S.E. 0000-0002-3124-066X; V.N.O. 0000-0001-6302-7137

Cite this article as: Chukwuma, I.F., Apeh, V.O., Nworah, F.N., Nwafor, F.I., Ezeanyika, L.U.S., & Ogugua, N. (2022). Protective effects of *Brenania brieyi* (De Wild) E.M.A.petit root bark fractions against inflammatory -mediated hemolysis and dyslipidemia in rats. *Istanbul Journal of Pharmacy,52*(3), 271-280. DOI: 10.26650/IstanbulJPharm.2022.1007696

ABSTRACT

Background and Aims: The inflammatory response, though protective, is the major cause of debilitating diseases when provoked excessively or if left unresolved. *Brenania brieyi* (De Wild) E.M.A.Petit is widely used in folk medicine for the treatment of inflammatory-related diseases. This study investigated the protective effects of methanol and chloroform root bark fractions of Brenania brieyi on inflammation-induced hemolysis and dyslipidemia.

Methods: Anti-inflammatory activity was investigated by inserting 20 mg of autoclaved cotton pellets into forty-five rats randomly distributed into nine groups (n=5), this excluded group 1 (baseline). The extent of hemolysis and dyslipidemia in the inflamed rats was ascertained from hematological parameters, lipid profile, and lipidemic index, while the possible underlying mechanisms of inflammation were determined using standard procedures.

Results: Treatment with varying doses of the root bark fractions of *B. brieyi* elicited a significant ($p< 0.05$) decrease in granuloma tissue and an increase ($p<0.05$) in hemoglobin, red and white blood cell count, packed cell volume, and platelets compared with the untreated group 2. A significant ($p<0.05$) decrease in cholesterol, triacylglycerols, and low-density lipoprotein, and a non-significant ($p>0.05$) increase in high-density lipoprotein were observed in almost all the test groups compared with group 2. There was a significant restoration of atherogenic and dyslipidemia indices and inhibition of acetic acid-induced vascular permeability, membrane hemolysis, and platelet aggregation in the fraction-treated groups compared with the control.

Conclusion: The findings from this study suggest that *B. brieyi* inhibits exudation and proliferation of granuloma-forming cells and also has the potential to restore the hematological parameters and lipid anomalies to their physiologic state under chronic inflammation. The possible mechanisms of its action could be inhibition of vascular permeability, stabilization of the membrane, or inhibition of platelet aggregation. This justifies the use of the plant in traditional medicine and also demonstrates its potential as a target for the discovery of new anti-inflammatory agents.

Keywords: Acute toxicity, Anti-inflammatory activity, *Brenania brieyi,* Chronic inflammation, Hematological parameters, Platelet aggregation, Lipid profile

Address for Correspondence:
Ifeoma Felicia CHUKWUMA, e-mail: chukwuma.ifeoma@unn.edu.ng

Submitted: 12.10.2021
Revision Requested: 04.01.2022
Last Revision Received: 09.09.2022
Accepted: 22.09.2022
Published Online: 30.12.2022

INTRODUCTION

An inflammatory response is a defense mechanism which eradicates noxious stimuli as well as initiating the tissue repair process (Kuum *et al*., 2018; Naher, Aziz, Akter, Rahman, & Sajon, 2019). In a bid to eliminate the injurious agent and restore tissue injury in the body, a network of mediators, cells, and pathways that send chemical signaling cascades are stimulated (Chen *et al.,* 2018; Altan *et al*., 2020). Paradoxically, when this response is not tightly controlled, regulated, or provoked excessively, it leads to inflammatory-induced diseases (Patil & Patil, 2017). Notably, the major predisposing factors to these diseases are not only the excessive generation of reactive oxygen and nitrogen species (Hwang & Thi, 2020), but more importantly, the provoked perturbation of blood homeostasis (Mosser, Hamidzadeh & Goncalves, 2021), and increased hydrolysis of polyunsaturated fatty acids, causing an increase in plasma lipids leading to dyslipidemia (Ogbe, Aghese &Abu, 2020). The increase in plasma lipids, especially triacylglycerols (TAG) and total cholesterol (TC), has been reported as a biomarker for the onset of cardiovascular diseases (CVD) (Aladaileh *et al*., 2019).

Due to the complex etiology of inflammation, the identification of effective therapeutic options has been a great challenge. Currently, in clinical settings, synthetic anti-inflammatory drugs such as steroidal and non-steroidal drugs are prescribed for the management of inflammatory-related diseases (Kuum *et al*., 2018; Alabi et al., 2019; Khan et al., 2021). Although these drugs transiently suppress symptoms and ameliorate inflammation, their chronic usage has severe adverse effects (Patil *et al*., 2019). Interestingly, recent studies have shown that a good percentage of the world's population relies solely on botanical preparations as medicine to meet their health needs (Fernandez-Moriano, Gomez-Serranillos & Crespo, 2016; Oloyede, Lukman & Salamu, 2020) in the management and treatment of numerous diseases, including inflammation (Majouli, Hamdi & Hlila*.,* 2017; Antonisamy et al., 2019; Majumder, Ghosh & Bhattacharya, 2020). So, in the milieu of the discovery of newer anti-inflammatory drug targets, natural products have remained of great interest (Rhetso, Seshadri, Ramnath, & Venkataramegowda, 2021) due to their better safety profile and lower cost in comparison to the increasing side effects and high cost of their synthetic counterparts (Kumar, Gupta & Singh*,* 2016; VasudhaUdupa et al., 2021).

A wide array of extensive studies have investigated the anti-inflammatory and anti-hemolytic effects of several plants on animal models (Dragomanova, Tancheva, Georgieva, & Klisurov, 2019; Patil *et al*., 2019), especially those with known folk remedies, but there is still a paucity of information about the potential of *B. brieyi* in the management of chronic inflammation-induced hemolysis and dyslipidemia. *Brieyi,* a member of the Rubiaceae family of flowering plants, is a herbal plant employed as a folk remedy in the management of several diseases, including swelling, infection, and endocrine disorders (Chukwuma, Nkwocha, Ezeanyika, & Ogugua, 2020a). In addition, a high abundance of phytoconstituents with reported anti-inflammatory activity such as squalene, hexadecenoic acids, 9-octadecanoic acids, eicosanoic acids, and pentadecanoic acids were identified in *B. brieyi* root bark (Odo, Ezeanyika, Ogugua, Joshua, & Okagu, 2017). Hence, this research was carried out to determine the protective effects of methanol and chloroform fractions of the root bark of *B. brieyi* against chronic inflammation in rats subjected to cotton pellet-induced inflammation, a model that could represent the proliferation of macrophages, fibroblasts, and neutrophils in human beings. The effects of the inflammatory cascade on hematological and lipid parameters, which reflect the extent of membrane stability, were ascertained. Additionally, the mechanisms underlying the fractions' actions were further investigated using acetic acid-induced permeability and membrane hemolysis inhibitory effects, as well as anti-platelet aggregatory tests.

MATERIAL AND METHODS

Collection and authentication of plant material

The *B. brieyi* root bark used for this study were collected from Njikoka, Anambra State, and identified by Mr. Felix Nwafor, a plant taxonomist in the Department of Pharmacognosy and Environmental Medicine. Voucher specimens with identification numbers PCG/UNN/0327 were deposited in his department's herbarium.

The procedure for extraction

The root barks of *B. brieyi* were dried at room temperature, pulverized, and extracted with chloroform and methanol in a ratio of 2:1 for 48 hours under cold maceration. It was filtered using filter paper (Whatman No. 4). The filtrate was later separated into two fractions by shaking it in 0.2 mL of distilled water. Using a separating funnel, the fractions were immediately separated into a methanol fraction of *B. brieyi* root bark (MFBB, upper layer) and a chloroform fraction of *B. brieyi* root bark (CFBB, lower layer). The MFBB and CFBB were then evaporated using a rotary evaporator at 45 °C. Both fractions were stored in a refrigerator at 4 °C.

Animals

Apparently healthy Swiss mice weighing 16.20 g ± 0.04 g and adult Wistar albino rats with an average weight of 120.11 ± 0.03 g, bought from the Animal House of the Faculty of Pharmaceutical Sciences, were used in this study. The animals were kept in a stainless steel cages in a 12 h light and dark cycle, 25 ±1 °C temperature, and given clean water and rodents' feed for at least two weeks before the procedure to acclimatize them to the environment. All the animals used were of different sexes, within a small age range, sourced from the same source, placed under the same environmental conditions, and fed the same rodent meal to eliminate confounding factors that might influence the results. This research work was done in conformity with all international and national approved guidelines on the care and use of laboratory animals as stated by the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, 1985). Ethical approval for the study was obtained from the Faculty of Biological Sciences Ethics and Biosafety Committee (Ref no: UNN/FBS/EC/1049).

Acute toxicity (LD_{50})

Acute toxicity and lethality studies of the fractions were determined using the method of Lorke (1983) using 36 mice distrib-

uted into twelve groups (six groups for each fraction) of three mice each being used for the first and second phases of the experiment. In the first phase, 3 sets of mice were orally administered 10, 100, and 1,000 mg/kg body weight of MFBB respectively via a cannula. Lethality and behavioral changes such as dizziness, irritation, jerking, and convulsion were observed for 24 h. This was followed by the administration of 1,600, 2,900, and 5,000 mg/kg b. w. of the same fraction for the other 3 sets in the second phase. Death and behavioral changes were also observed for 24 h after the administration of the test substance. The same procedure was also used to determine acute toxicity for CFBB.

LD_{50} of each extract was calculated using this formula:

Where Do= highest dose that gave no mortality

D100= lowest dose that produced mortality.

Cotton pellet-induced chronic inflammatory model

A total of 45 male Wistar rats were randomly grouped into nine groups of five rats each and were implanted with 20 mg of autoclaved cotton pellets according to the method of Mosquera et al. (2011) with the exception of group 1, which served as the baseline. Group 2 was administered normal saline, group 3 was treated with indomethacin (10 mg/kg body weight), groups 4-6, and groups 7-9 received 50, 100, and 200 mg/kg b. w. of MFBB and CFBB, respectively, for seven days. The animals were sacrificed on the eighth day after being anesthetized with chloroform. Blood samples were collected through cardiac puncture by the principal investigator, after which the pellets were carefully removed, dried in an oven at 60 °C for 24 h, and weighed. The blood samples were used for the determination of hematological parameters and lipid profiles. The change in granuloma tissue weight was calculated as follows:

The final weight of the pellet - the initial weight of the pellet.

Determination of hematological parameters from the serum of rats implanted with a cotton pellet

Blood samples used for measurement of hematological parameters were transferred into EDTA (anticoagulant) bottles and used immediately to measure the full blood count using a hematology analyzer (Erma PCE 210, Japan).

Determination of lipid profile from the serum of rats implanted with a cotton pellet

The following procedures were used to determine the lipid profile: Total cholesterol by the Allain, Poon, Chan, Richmond, & Fu, 1974 method using Quimica Clinical Aplicada (QCA) commercial kits, triacylglycerols was determined with the Randox commercial kit using the method of Albers, Warnick & Chenng (1978), HDL was measured with Quimica Clinical Aplicada (QCA) commercial kits using Albers et al. (1978) methods, while the polyvinyl sulphate method was used to determine the LDL.

Estimation of atherogenic/dyslipidemia indices

The following equations were used to calculate the atherogenic/dyslipidemia indices as described by Ogbe et al. (2020).

a. Cardiac risk ratio (CRR) = $\frac{\text{Total cholesterol}}{\text{HDL}}$

b. Atherogenic coefficient (AC) = $\frac{\text{Total cholesterol-HDL}}{\text{HDL}}$

c. Classical ratio (CR) = $\frac{\text{LDL}}{\text{HDL}}$

d. Atherogenic index of plasma (AIP) = $\log \frac{\text{Triglyceride}}{\text{HDL}}$

Mechanisms of inflammatory reactions

The following mechanisms of anti-inflammatory activity were investigated: Acetic acid-induced vascular permeability test according to Whittle (1964), the extent of membrane stability by Shinde *et al*. (1999), and anti-platelet aggregatory activity was determined using the Born & Cross (1963) method. The percentage inhibition of the test substances (fractions/ and standard drug) were calculated relative to the control as shown in equations below:

a. Inhibition of vascular permeability (%) = $\left[\frac{AC-AT}{AC} \text{ X } 100\right]$

Where: AC = Absorbance of the control while AT = Absorbance of the fractions/test drug.

b. inhibition of membrane hemolysis (%) = $1 - \frac{OD2-OD1}{OD3-OD1} \text{ X } 100$

Where OD_1 = absorbance of test sample unheated, OD_2 = absorbance of test sample heated, and OD_3 = absorbance of control sample heated.

c. Inhibition of platelet aggregation (%) =

Where : AT = Absorbance of the fractions / test drug while AC = Absorbance of the control.

Statistical analysis

The statistical package for social science (SPSS) for windows version 23 (SPSS Inc., Chicago, IL, USA) was used to analyze the data obtained using one-way analysis of variance (ANOVA), and Tukey's *post hoc* test. $p < 0.05$ was taken as the significant threshold. The results were presented as means ± standard deviation.

RESULTS

Acute toxicity study (LD_{50})

There were no observed behavioral changes or lethality in mice administered 10-1,600 mg/kg b. w of each fraction after 24 h, while sedation, weakness, and dullness were observed in mice given 2,900 and 5,000 mg/kg b. w. of both fractions. Moreover, death was recorded in mice that received 5,000 mg/kg body weight of each fraction within 24 h of administration (Table 1).

Effects of MFBB and CFBB on cotton pellet-induced granuloma tissue formation

Cotton pellet-induced formation of granuloma tissue was inhibited in groups 3-9 treated with different doses of the fractions and indomethacin. However, groups 4 and 6 treated with MFBB exhibited a significantly ($p < 0.05$) higher weight of granuloma tissue compared with groups 7 and 9 given the same dose of CFBB (Figure 1).

Results are presented as mean ± SD (n = 5). Mean values having '*' denotes significant difference at $p < 0.05$ compared with negative-control (normal saline).

Table 1. Acute toxicity study (LD_{50}) of the root bark fraction of *B. brieyi*.

Dose in mg/kg body weight	Number of deaths recorded with MFBB	Number of deaths recorded with CFBB
Phase 1		
10	0/3	0/3
100	0/3	0/3
1000	0/3	0/3
Phase 2		
1600	0/3	0/3
2900	0/3	0/3
5000	1/3	1/3

n=3

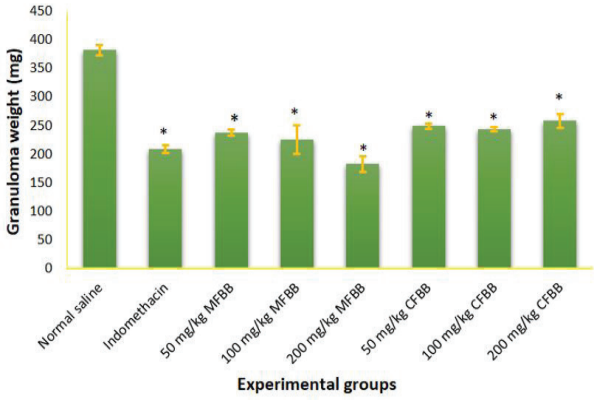

Figure 1. Changes in the weight of granuloma tissue formed after treatment.

Effects of MFBB and CFBB on hematological parameters of rats implanted with a cotton pellet

A significant ($p < 0.05$) decrease in Hb, RBC, WBC, and PCV with a resultant increase in platelet count was observed in group 2 (given normal saline) after cotton pellet implantation compared with group 1. Interestingly, a significant ($p < 0.05$) concentration-dependent restoration of Hb, RBC, WBC, and platelet count occurred in rats administered varying doses of the fractions of the root bark of *B. brieyi* and indomethacin. Varied doses of MFBB were efficacious in restoring RBC, WBC, and platelet counts, compared with CFBB, but the reverse was the case with Hb and PCV (Table 2).

Key:

Group 1: Normal rats not implanted with cotton pellets (baseline).

Group 2: Cotton pellet + treatment with 1 ml/kg body weight of normal saline (negative control).

Group 3: Cotton pellet + treatment with 10 mg/kg body weight of indomethacin (standard drug).

Groups 4, 5, and 6 rats were treated with 50, 100, and 200 mg/kg body weight of MFBB, respectively, after cotton pellet implantation.

Groups 7, 8, and 9 were treated with 50, 100, and 200 mg/kg body weight of CFBB, respectively, after cotton pellet implantation.

Effects of MFBB and CFBB on lipid profile indices of rats implanted with a cotton pellet

Implantation of cotton pellets significantly ($p < 0.05$) altered the lipid profile indices in group 2 when compared with group 1. Interestingly, groups 3, 4, 5, and 6, treated with indomethacin (50 mg/kg) and MFBB (50, 100, and 200 mg/kg) respectively, had a significant ($p < 0.05$) decrease in cholesterol, TAG, and LDL when compared with group 2, except in high-density lipoprotein, which showed a significant ($p < 0.05$) increase only in groups 3 and 6. Moreover, only 200 mg/kg b. w of CFBB was effective in attenuating all the lipid indices significantly ($p < 0.05$) compared with group 2. The MFBB was found to be more potent in decreasing cholesterol and LDL compared with CFBB, whereas the reverse was the case with TAG (Figure 2).

Table 2. Effects of MFBB and CFBB on concentrations of some serum hematological parameters of rats implanted with a cotton pellet.

Groups	Hb (g/dl)	RBC (x 10^6/l)	WBC (x10^3/l)	PCV (%)	Platelets (x10^6/l)
1	25.14±2.22	5.44 ±1.37	9560.00±219.09	45.20±5.63	135.00±9.35
2	13.24±1.23#	3.36 ±1.08#	7860.00±219.09#	25.00±3.67#	110.00±14.58#
3	22.44±2.09*	5.24 ±1.75*#	6760.00±167.33*#	48.00±2.92*	116.00±12.94#
4	19.70± 1.08*#	4.34 ±0.58*#	9120.00±228.04*#	34.20±4.60*#	198.00±12.55*#
5	22.38±2.45*	5.00 ±1.12*#	12440.0±260.77*#	36.80±5.54*#	169.00±4.18*#
6	22.94±0.36*	6.02 ±2.74*#	14480.0±109.54*#	44.20±3.49*	125.00±6.12*
7	23.40±1.27*	3.68 ±1.84*#	8280.00±109.54*#	40.20±5.35*	133.00±12.04*
8	23.68±1.61*	4.72 ±1.18*#	10040.0±167.33*#	44.80±3.27*	150.00 ±7.90*#
9	24.88±1.18*	5.24 ±1.58*#	9720.00± 178.89*	46.00±0.71*	113.00±12.55*#

Results are presented as mean ± SD (n=5). Mean values with '#' denotes significant difference at $p < 0.05$ compared with baseline while '*' denotes significant difference at $p<0.05$ compared with negative-control.

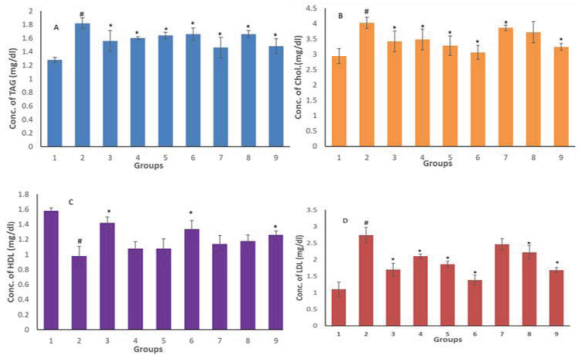

Figure 2. Effects of root barks fractions of *B. brieyi* on concentrations of serum lipid profile indices of rats implanted with cotton pellet.

TAG (A), Chol. (B), HDL (C) and LDL (D) stands for triacylglycerol, cholesterols, high density lipoprotein and low density lipoprotein respectively. Values are presented as mean ± SD (n = 5). Mean values with '#' denotes significant difference ($p < 0.05$) compared with baseline while '*' denotes significant difference ($p < 0.05$) compared with negative-control.

Key:

Group 1: Normal rats not implanted with cotton pellets (baseline).

Group 2: Cotton pellet + treatment with 1 ml/kg body weight of normal saline (negative control).

Group 3: Cotton pellet + treatment with 10 mg/kg body weight of indomethacin (standard drug).

Groups 4, 5, and 6 rats were treated with 50, 100, and 200 mg/kg body weight of MFBB, respectively, after cotton pellet implantation.

Groups 7, 8, and 9 were treated with 50, 100, and 200 mg/kg body weight of CFBB, respectively, after cotton pellet implantation.

Effects of MFBB and CFBB on atherogenic /dyslipidemia indices in rats

There was an increase in CRR, AC, CR, and AIP in groups implanted with cotton pellets compared with group 1 (normal rats). Interestingly, significant dose-dependent restoration of dyslipidemia was observed in almost all the fractions treated groups when compared with the untreated control (group 2). The inhibitory effects of the standard drug were found to be comparable with groups administered 200 mg/kg b. w of both fractions. However, the highest inhibitory effects of cardiac risk ratio (CRR), atherogenic coefficient (AC), and classical ratio (CR) were recorded in group 6 administered 200 mg/kg b. w of MFBB (Table 3).

Key:

Group 1: Normal rats not implanted with cotton pellets (baseline).

Group 2: Cotton pellet + treatment with 1 ml/kg body weight of normal saline (negative control).

Group 3: Cotton pellet + treatment with 10 mg/kg body weight of indomethacin (standard drug).

Groups 4, 5, and 6 rats were treated with 50, 100, and 200 mg/kg body weight of MFBB, respectively, after cotton pellet implantation.

Groups 7, 8, and 9 were treated with 50, 100, and 200 mg/kg body weight of CFBB, respectively, after cotton pellet implantation

Effects of MFBB and CFBB on acetic acid-induced vascular permeability in rats

A significant inhibition of vascular permeability, which was in a dose-dependent manner, was observed in the rats administered with both fractions. The inhibitory effect (72%) of group 6, given 200 mg/kg of CFBB, was significantly ($p < 0.05$) higher compared with the 69.3% inhibition observed in group 4, given the same dose of MFBB. However, the percentage inhibition (78%) of vascular permeability in the group given the standard drug, indomethacin, was significantly higher compared with

Table 3. Effects of MFBB and CFBB on atherogenic/dyslipidemia indices in rats implanted with a cotton pellet.

Groups	Atherogenic/dyslipidemia indices in rats			
	CRR	AC	CR	AIP
1	1.86 (54)*	0.86 (72)*	0.70 (75)*	0.09 (133)*
2	4.10 (-)	3.10 (-)	2.80 (-)	0.27 (-)
3	2.41 (41)*	1.41 (55)*	1.20 (57)*	0.04 (85)*
4	3.22 (21)*	2.22 (28)*	1.94 (31)*	0.17 (37)*
5	3.04 (26)*	2.04 (34)*	1.72 (39)*	0.18 (33)*
6	2.28 (44)*	1.28 (59)*	1.03 (63)*	0.09 (67)*
7	3.39 (17)	2.39 (22)	2.16 (23)*	0.11 (59)*
8	3.15 (23)*	2.15 (30)*	1.88 (33)*	0.15 (44)*
9	2.57 (37)*	1.57 (49)*	1.33 (53)*	0.07(74)*

Values are presented as the mean of 5 rats. Percentage changes (%) in mean values were calculated relative to control and enclosed in parenthesis. Values with * are significantly ($p < 0.05$) different from control.
CRR, AC, CR, and AIP denote cardiac risk ratio, atherogenic coefficient, classical ratio, and atherogenic index power respectively.

all the groups administered with different doses of both fractions (Table 4).

Effects of MFBB and CFBB on heat-induced hemolysis of human red blood cells

The fractions inhibited heat-induced hemolysis of RBC in a reverse concentration-dependent manner. However, MFBB provoked a significantly ($p < 0.05$) higher membrane stabilization potential across all concentrations assayed compared with CFBB and indomethacin (Table 5).

Effects of root barks fractions of *B brieyi* on platelet aggregation induced by $CaCl_2$

Both fractions inhibited *in vitro* platelet aggregation induced by $CaCl_2$ in a manner comparable with the standard drug, indomethacin. The highest anti-aggregatory activity was recorded at the highest time assayed (150 sec.). The results in Table 6 also reveal that 200 and 400µg/ml of indomethacin reduced platelet aggregatory response to $CaCl_2$ in a concentration and time-dependent manner. The inhibition of platelet aggregation by the plant was comparable with that of indomethacin.

Table 5. Effects of MFBB and CFBB on heat-induced hemolysis of human red blood cells.

Treatments	Conc. (µg/ml)	% inhibition of HRBC hemolysis
Control	-	0
MFBB	100	89.08
	200	84.62
	400	86.32
	600	87.12
	800	83.92
CFBB	100	74.76
	200	71.86
	400	64.36
	600	61.65
	800	61.96
Indomethacin	200	75.36
	400	77.46

Results of reported as mean ± SD of triplicate absorbance determination. The inhibition of HRBC hemolysis (%) was calculated relative to control.

DISCUSSION

The extent of exudation and proliferation as a result of tissue degeneration and fibrosis under chronic inflammatory response is measured by the cotton pellet-induced granuloma model (Kumar et al., 2016; Misra, Varma & Kumar, 2018). Hence, inhibition of granuloma formation by the fractions suggests its potency in offering protection against chronic inflammation, which is recognized as the predisposing factor for the pathogenesis of various forms of cancer (Patil et al., 2019). This suggests that the fractions inhibited abnormal permeability of the vascular tissues and mobilization of inflammatory cells and mediators. It could be possible that the bioactive components present in the plant, which demonstrated antioxidant and anti-inflammatory properties (Chukwuma et al., 2020b; Chukwuma et al., 2021), inhibited the release of inflammatory mediators such as prostaglandin, thereby hindering proliferation of granuloma-forming cells like macrophages, fibroblasts, and neutrophils (Kumar et al., 2016). Lending credence to this also is the high phenolic content found in the plant, which has been reported to inhibit the expression of pro-inflammatory genes (Chukwuma et al., 2020a).

A prolonged inflammatory response is associated with modification of hematological parameters. Hence, monitoring hematological indices under chronic inflammatory diseases helps to ascertain the extent of tissue damage. The observed decreases in Hb, PCV, and RBC counts in groups implanted with cotton pellets reflect the presence of anemia. Also, the excess release of ROS in inflammatory reactions degrades hemoglobin and lipid components of cells. Interestingly, the significant restoration of Hb, RBC, WBC, and PCV in groups treated with the fractions when compared with group 2 might be due to antioxidative compounds found in the fractions which inhibited the release of ROS (Odo et al., 2017; Chukwuma et al., 2020a; Chukwuma et al., 2020b) and hence, preserved the integrity of the cell membrane from hemolysis of RBC. This concurs with the report of Haddouchi, Chaouche, Saker, Ghellai, & Boudjemai (2021) who also studied the antioxidant potential of polyphenolic compounds. Moreover, the increase in WBC count suggests the potential of the plant in maintaining the integrity of the rats' immune system while the observed increase in

Table 4. Percentage inhibition of vascular permeability by MFBB and CFBB.

Groups	Treatments	Dosage (mg/kg)	Absorbance (610 nm)	Inhibition (%)
1	Control	-	0.274 ± 0.006	0
2	Indomethacin	50	0.059 ± 0.005*	78.47
3	MFBB	100	0.128 ± 0.006*	53.28
4		200	0.084 ± 0.003*	69.34
5	CFBB	100	0.144 ± 0.003*	47.45
6		200	0.076 ± 0.004*	72.26

Results are presented as mean ± SD n = 5. The absorbance of the treatment groups was used to calculate % inhibition relative to control. Absorbance with '*' is significantly different ($p < 0.05$) compared with the control.

Table 6. Effects of MFBB and CFBB on platelet aggregation induced by $CaCl_2$.

Conc. (µg/ml)	% inhibition of platelet aggregation at different time intervals					
	0s	30s	60s	90s	120s	150s
MFBB						
100	50.37 ± 4.01	50.00± 3.20	50.13 ±2.71	50.52 ± 3.21	50.53± 2.34	50.53 ± 2.17
200	48.97 ± 3.75	48.39 ± 2.89	48.52 ± 4.50	48.64 ± 2.90	48.63± 2.10	48.77± 4.00
400	42.49 ± 5.31	42.34 ± 3.42	42.47± 2.01	42.90 ± 1.78	42.68± 1.90	42.64± 1.21
600	17.08 ± 2.65	15.04 ± 1.87	15.49± 3.42	15.63 ± 2.92	17.55± 2.17	15.00± 3.10
800	28.67 ± 1.09	38.02 ± 3.40	27.38 ± 3.11	27.59 ± 3.12	27.41± 3.20	27.51 ± 4.12
CFBB						
100	5.69 ± 0.21	7.25 ± 1.23	7.23 ± 0.98	7.35 ± 2.96	6.93± 0.18	6.03± 0.97
200	24.05 ± 2.47	21.95 ± 2.00	21.72 ± 3.17	22.22 ± 1.32	21.99± 3.19	21.43± 4.12
400	34.11 ± 3.33	33.33 ± 1.34	33.45 ± 2.00	33.92 ± 3.45	34.27± 2.14	34.15± 2.93
600	48.71 ± 4.52	49.07 ± 2.05	49.20 ± 1.15	49.33 ± 5.10	49.46± 3.71	49.46± 1.87
800	56.46 ± 2.90	57.05 ± 3.11	54.63 ± 5.12	57.34 ± 0.78	57.37± 2.09	57.40 ± 3.12
INDO						
400	34.96 ± 4.53	30.93 ± 2.18	27.65 ± 1.26	27.02 ± 1.23	26.56± 3.11	25.49 ± 1.23
600	47.76± 3.90	41.46 ± 4.57	36.96 ± 2.13	36.15 ± 2.30	35.17± 1.90	35.51 ± 2.17

Indo. Stands for indomethacin. Percentage inhibition of platelet aggregation was calculated relative to control.

platelets suggests its wound healing properties since platelets are known to be involved in the healing of damaged tissues (Anyasor, Okanlawon & Ogunbiyi, 2019).

A chronic inflammatory response activates acute phase proteins that alter lipid metabolism, resulting in a decrease in HDL, impairment of reverse cholesterol transport, changes in apolipoproteins, and changes in cholesterol efflux regulatory proteins (Essawy, Abo-elmatty, Ghazy, Badr, & Sterner, 2014; Esteve, Ricart, & Fernández-Real, 2005). Normalization of these lipid anomalies in this study was demonstrated by the decreases in total cholesterol, TAG, and LDL and an increase in HDL after treatment. This potency could be attributed to the antioxidant compounds identified in the plant in the preliminary studies by Odo et al. (2017) which previous studies have reported to be antioxidant molecules (Chakraborty et al., 2021). Notably, squalene found in the fraction is a cardioprotective agent, an enhancer of WBC, and increases fecal excretion which reduces the concentration of cholesterol (Odo et al. 2017). Also, the most abundant compound found in the fraction, 9-octadecanoic acid (oleic acid), helps in preventing atherosclerosis due to its efficacy in lowering LDL (Nkwocha, Odo & Umeakuana, 2019). In the same vein, a previous study by Chukwuma et al. (2021) demonstrated the phospholipase A2 inhibitory effects of the plant, which prevents the breakdown of the lipid membrane. So, this suggests that MFBB and CFBB could be very helpful in reducing the onset of cardiovascular disease since studies have shown that a significant lowering of LDL-cholesterol and a rise in HDL-C are reliable biochemical biomarkers for the prevention of atherosclerosis and ischemic conditions (Ikumawoyi, Awodele, Rotimi, & Fashina, 2016).

Emerging evidence has shown that chronic inflammatory diseases orchestrate the atherosclerotic vasculopathy involved in the pathophysiology of cardiovascular diseases (CVD) (Acay et al., 2014). The use of lipid profiles alone to determine the prevalence and severity of CVD has been questioned. Hence, the use of atherogenic/dyslipidemia indices, mainly atherogenic index of plasma (AIP), which estimate the balance between atherogenic and other non-atherogenic factors, has proven to be a better predictor of CVD than lipid profile (Acay et al., 2014; Ogbe et al., 2020). The observed decrease in CRR, AC, CR, and AIP in this study suggests ameliorating effects of the fractions in averting inflammatory-induced dyslipidemia. The decrease in atherogenic/dyslipidemia indices in this study could be attributed to a decrease in LDL and an increase in HDL. Lipids accumulate in macrophages during inflammation to form lipid foam cells. These cells form fatty streaks when they accumulate in the walls of the arteries, causing atherosclerotic plague (Esteve, Ricart, & Fernández-Real, 2005). Agents that subvert infiltration of inflammatory cells into the adipose tissues help to prevent excessive production of cytokines and adipose lipids which potentiate these lipid metabolism disorders (Esteve *et al*., 2004).

The release of immune cells and mediators in the presence of a stimulus dilates the blood vessels to enhance the mobilization of vascular components to the inflamed region (Chen *et al*., 2018; Altan *et al*., 2020). This study investigated the inhibitory effects of MFBB and CFBB on a vasodilator, acetic acid. Acetic acid stimulates mast cells, which enhances the release of inflammatory agents responsible for dilating blood vessels such as prostaglandins, histamine, serotonin, bradykinin, and leukotrienes (Kumar *et al*., 2016; Patil *et al.,* 2019). The observed inhibition of vascular permeability by MFBB and CFBB suggests

that they could suppress the exudative phase of inflammation, which would avert tissue damage. This potency could also be due to its inhibitory effect on phospholipase A2 and prostaglandin synthase, which hinders the release of inflammatory mediators including A2, PGD2, PGE2, and PG12, involved in vaso-dilation (Chukwuma et al., 2021). Perhaps the bioactive compounds found in the fractions stabilize cell membranes, as shown in their high inhibition of heat-induced membrane stabilization study. Conversely, the plants ability to inhibit membrane hemolysis may be due to their high antioxidant activity, as demonstrated in the plants' *in vitro* and *in vivo* antioxidant studies (Chukwuma et al., 2020a, Chukwuma et al., 2020b). Plants with antioxidant activity have been reported to be key anti-inflammatory drug targets since they prevent the leakage of fluid into the peritoneum, thereby suppressing inflammation. This will also avert the biochemical cascade involved in chronic inflammation, such as granuloma tissue formation.

Furthermore, an increase in thromboxane and platelet-activating factor production, which causes aggregation of platelets, is a marker of inflammation and a pharmacological target in the management of inflammatory diseases (Sokeng, Rokeya, Hannan, Ali, & Kamtchouing, 2013; Gros, Ollivie & Ho- Tin-Noe, 2014). Platelet aggregation helps in cellular hemostasis. However, excessive aggregation of platelets in inflammatory reactions leads to thrombotic diseases (Chukwunelo et al., 2019). Interestingly, both MFBB and CFBB had anti-platelet aggregatory capacity. Platelet aggregation can be inhibited through the inactivation of intracellular signaling pathways or by blocking membrane receptors (Mykola, Ganna & Gennadiy, 2015). This suggests that the fractions inhibited activation of COX-1, thereby limiting the synthesis of thromboxane and platelet-activating factors from arachidonic acid. Hence, the fractions could help in circumventing factors that predispose one to chronic inflammation-induced diseases such as cardiovascular diseases.

CONCLUSIONS

The results of this study show that the root bark fractions of *B. brieyi* inhibited the exudation and proliferation of granuloma-forming cells, thereby limiting the formation of granuloma tissues. It also demonstrated the potential to inhibit hemolysis and hyperlipidemic aberrations of blood cells and membrane lipids, which could be responsible for normalizing hemolytic and hyperlipidemic anomalies in inflamed rats. This could be due to its ability to inhibit vascular permeability, membrane hemolysis, and platelet aggregation, limiting excessive infiltration of inflammatory exudates that can cause membrane peroxidation and cell damage. This suggests that it has anti-inflammatory activity and efficacy in restoring body homeostasis under inflammation. This justifies the use of the plant in traditional medicine for the management of inflammatory diseases and also opens the window for its usage as a target for the discovery of new anti-inflammatory agents.

Peer-review: Externally peer-reviewed.

Informed Consent: Written consent was obtained from the participants.

Author Contributions: Conception/Design of Study- I.F.C., L.U.S.E., V.N.O.; Data Acquisition-I.F.C., V.O.A., F.I.N.; Data Analysis/Interpretation- I.F.C., V.O.A., F.N.N., L.U.S.E.; Drafting Manuscript- I.F.C., V.O.A., F.N.N., F.I.N.; Critical Revision of Manuscript- L.U.S.E.; Final Approval and Accountability I.F.C., V.O.A., F.N.N., L.U.S.E.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared no financial support.

Acknowledgement: The authors are highly grateful to Mr. Chukwuma Odo, who provided funds used for the research work.

REFERENCES

- Acay, A., Ulu, M.S., Ahsen, A., Ozkececi, G., Demir, K., Ozuguz, U., … Acarturk, G. (2014). Atherogenic index as a predictor of atherosclerosis in subjects with familial Mediterranean fever. *Medicina,* 50(6), 329-333. https://doi.org/10.1016/j.medici.2014.11.00.
- Alabi, A.O., Ajayi, A.M., Omorogbe, O. & Umukoro, S. (2019). Antinociceptive and anti-inflammatory effects of the aqueous extract of blended leaves of *ocimumgratissium* and *psidium guajava*. *Clinical Phytoscience*, 5, 34-42. https://doi.org/10.1186/s40816-019-0130-2.
- Aladaileh, S.H., Saghir, S.A.M., Murugesu, K., Sadikun, A., Ahmad, A., Kaur, G., … Murugaiyah V (2019). Antihyperlipidemic and antioxidant effects of *Averrhoa carambola* extract in high-fat diet-fed rats. *Biomedicines*, 7, 72-93. Doi:10.3390/biomedicines7030072.
- Albers, J.J., Warnick, G.R., Chenng, M.C. (1978). Quantitation of high-density lipoproteins. *Lipids*, 13, 926-932. https://doi.org/10.1007/BF02533852
- Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. & Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry,* 20, 470-475. DOI:10.1093/clinchem/20.4.470.
- Altan, A., Balci, Y.H., Karataş, O., Taşkan, M.M., Gevrek, F., Çolak, S. & Akbulut, N. (2020). Free and liposome form of gallic acid improves calvarial bone wound healing in Wistar rats. *Asian Pacific Journal of Tropical Biomedicine*, 10, 156-163.Doi**:** 10.4103/2221-1691.280297.
- Antonisamy, P., Agastian, P., Kang, C-W., Kim, N. & Kim. J-H. (2019). Anti-inflammatory activity of rhein isolated from the flower of Cassia fistula L. And possible underlying mechanisms. Saudi Journal of Biological Sciences, 26, 96-104. Doi: 10.1016/j.sjbs.2017.04.011.
- Anyasor, G.N., Okanlawon, A.A. & Ogunbiyi, B. (2019). Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. *Clinical Phytosciences*, 5, 49-61. https://doi.org/10.1186/s40816-019-0137-8.
- Born, G.V.R. & Cross, M.J. (1963). Inhibition of aggregation of blood platelets by substances related to adenosine diphosphate. *The Journal of Physiology,* 166, 29-30. Doi: 10.1113/jphysiol.1963.sp007185.
- Chakraborty, S., Majumder, S., Ghosh, A., Saha, S. & Bhattacharya, M. (2021). Metabolomics of potential contenders conferring antioxidant property to varied polar and non-polar solvent extracts of Edgaria darjeelingensis C. B.Clarke. *Bulletin of National Research Centre,* 45, 48-59. https://doi.org/10.1186/s42269-021-00503-3.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., … Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget,* 9, 7204-7218. Doi: 10.18632/oncotarget.23208.
- Chukwuma, I.F., Nkwocha, C.C., Ezeanyika, L.U.S. & Ogugua, V.N. (2020a). Phytochemical Investigation and *In Vitro* Antioxidant Potency of Root Bark of *Brenania brieyi* fractions. *Tropical Journal of Natural Products Research*, 4(11), 970-975. doi.org/10.26538/tjnpr/v4i11.21.

- Chukwuma, I.F., Apeh, V.O., Ezeanyika, L.U.S. & Ogugua, V.N. (2020b). B*renania brieyi* root bark extracts ameliorate chronic inflammation-mediated oxidative stress in Wistar rats. Doi:10.3390/cahd2020-08556.
- Chukwuma, I.F., Apeh, V.O., Nworah, F.N., Nkwocha, C.C., Emaimo, J., Ogugua, V. N. & Ezeanyika, L. U. S. (2022). Inhibition of phospholipase A2 and prostaglandin synthase activities as possible mechanistic insight into the anti-inflammatory activity of Brenania brieyi methanol and chloroform fractions. Thai Journal of Pharmaceutical Sciences. 2022;46(1):75-84 .
- Chukwunelo, A.C., Anosike, A.C., Nnamonu, E.I., Ekpo, D.E., James, P.O. & Okonkwo, T.I. (2019) Evaluation of Leukocyte Mobilization and Platelet Aggregatory Effects of Ciprofloxacin, Lincomycin and Erythromycin in Wistar Albino Rats. *Notulae Scientia Biologicae*, 11(4), 345-351. DOI: 10.15835/nsb11410491.
- Dragomanova, S., Tancheva, L., Georgieva, M. & Klisurov, R. (2019). Analgesic and anti-inflammatory activity of monoterpenoid Myrtenal in rodents. *Journal of IMAB,* 25, 2406-2413. https://doi.org/10.5272/jimab.2019251.2406.
- Essawy, S.S., Abo-elmatty, D.M., Ghazy, N.M., Badr, J.M. & Sterner, O. (2014). Antioxidant and anti-inflammatory effects of *Marrubium-alysson* extracts in high cholesterol-fed rabbits. *Saudi Pharmaceutical Journal,* 22, 472-482. Doi: 10.1016/j.jsps.2013.12.004.
- Esteve, E., Ricart, W. & Fernández-Real, J.M. (2005). Dyslipidemia and inflammation: An evolutionary conserved mechanism. *Clinical Nutrition,* 24(1), 6–31. doi: 10.1016/j.clnu.2004.08.004.
- Fernandez-Moriano, C., Gomez-Serranillos, M.P. & Crespo, A. (2016). Antioxidant potential of lichen species and their secondary metabolites. A systematic review. *Pharmaceutical Biology*, 54, 1-17. https://doi. 10.3109/13880209.2014.1003354.
- Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18, 499-502. https://doi.org/10.1093/clinchem/18.6.499.
- Gros, A., Ollivier, V. & Ho- Tin-Noe, B. (2014). Platelets in inflammation: Regulation of leukocyte activities and vascular repair. *Frontiers in Immunology*, 5, 678-685. Doi: 10.3389/fimmu.2014.00678.
- Haddouchi, F., Chaouche, T. M., Saker, M., Ghellai, I. & Boudjemai, O. (2021). Phytochemical screening, phenolic content and antioxidant activity of Lavandula species extracts from Algeria. *İstanbul Journal of Pharmacy*, 51 (1), 111-117. https://dergipark.org.tr/en/pub/iujp/issue/62349/938874.
- Hwang, E.S. & Thi, N.D. (2020). Antioxidant and anti-inflammatory activities of Orostachys japonicus. *Asian Pacific Journal of Tropical Biomedicines*, 10, 516-522. doi: 10.4103/2221-1691.294092.
- Ikumawoyi, Y.O., Awodele, O., Rotimi, K. & Fashina, A.Y. (2016). Evaluation of the effects of the hydro-ethanolic root extract of *zanthoxylum zanthoxyloides* on hematological parameters and oxidative stress in cyclophospamide treated rats. *African Journal of Traditional Complementary and Alternative Medicine*, 13, 153-159. Doi: 10.21010/ajtcam.v13i5.20.
- Khan, F., Magaji, M. G., Abdu-Aguye, I., Hussaini, I. M., Hamza, A., Olarukooba, A.B. Sani, M.A. & Maje, I. M. (2021). Phytochemical profiling of the bioactive principles of Alysicarpus glumaceus (Vahl) DC. aerial parts. *İstanbul Journal of Pharmacy*, 51(2), 228-238. DOI: 10.26650/IstanbulJPharm.2020.0071.
- Kumar, R., Gupta, Y.K. & Singh, S. (2016). Anti-inflammatory and anti-granuloma activity of *Berberis aristata* DC. in experimental models of inflammation**.** *Indian Journal of Pharmacology*, 48, 155-161. Doi 10.4103/0253-7613.178831.
- Kuum, M., Guemmogne, R., Ndzana, M., Tchadji, J., Lissom, A. & Dimo, T. (2018). Anti-inflammatory effects of the stem barks from Albizia *ferruginea* (Mimosaceae) on chronic inflammation induced in rats. International *Journal* of *Innovative* Research in *Medical Science*, 3, 2183-2195. Doi: 10.23958/ijirms/vol03-i09/423.
- Lorke, D. (1983). Determination of acute toxicity. *Archives of Toxicology,* 53, 275-287. Doi:10.1007/bf01234480.
- Majouli, K., Hamdi, A. & Hlila, M.B. (2017). Phytochemical analysis and biological activities of *Hertiacheirifolia* L. roots extracts. *Asian Pacific Journal of Tropical Medicines,* 10:1134-1139. https://doi.org/10.1016/j.apjtm.2017.10.020.
- Majumder, S., Ghosh, A. & Bhattacharya, M. (2020). Natural anti-inflammatory terpenoids in Camellia japonica leaf and probable biosynthesis pathways of the metabolome. *Bulletin of National Research Center*, 44, 141-151. https://doi.org/10.1186/s42269-020-00397-7.
- Misra, A.K., Varma, S.K. & Kumar, R. (2018). Anti-inflammatory effect of an extract of Agave Americana on experimental Animals. Pharmacognosy Research, 10, 104-108. DOI: 10.4103/pr.pr_64_17.
- Mosser, D.M., Hamidzadeh, K. & Goncalves, R. (2021). Macrophages and the maintenance of homeostasis. *Cellular and Molecular Immunology*, 18, 579–587. https://doi.org/10.1038/s41423-020-00541-3.
- Mosquera, D.M.G., Ortega, Y.H., Kilonda, A., Dehaen, V., Pieters, L. & Apers, S. (2011). Evaluation of the in vivo anti-inflammatory activity of a flavonoid glycoside from Boldoa purpurascens. *Phytochemistry Letters*, 4, 231-234.
- Mykola, V., Ganna, S. & Gennadiy, T. (2015). Hematological abnormalities in Ukrainian patients with rheumatoid arthritis. *Journal of Arthritis*, 4, 146-148. DOI: 10.4172/2167-7921.1000146.
- Naher, S., Aziz, M., Akter, M., Rahman, S. & Sajon, S. (2019). Analgesic, anti-inflammatory and anti-pyretic activities of methanolic extract of *Cordyline fruticosa* (L.) Chev. leaves. *Journal of Research in Pharmacy*, 23, 198-207. https://doi.org/10.12991/jrp.2019.125.
- Nkwocha, C.C., Odo, I.F. & Umeakuana, C.D. (2019). Fatty Acid Profile of Some Selected Locally Consumed Vegetable Oils in Enugu State, Nigeria. *America journal of food Science and Nutrition*, 7, 130-135. http://pubs.sciepub.com/ajfn/7/4/3.
- Odo, I.F., Ezeanyika, L.U.S., Ogugua, V.N., Joshua, P.E. & Okagu, I.U. (2017). FTIR and GC-MS spectroscopic analysis of methanol and chloroform extracts of *Brenania brieyi* root bark. *American Journal* of *Research Communication*, 5, 44-54.
- Ogbe, R.J., Agbese, S.P. & Abu, A.H. (2020). Protective effect of aqueous extract of Lophira *lanceolata* leaf against cisplatin-induced hepatorenal injuries and dyslipidemia in Wistar rats. *Clinical Phytosciences*, 6, 4-14. https://doi.org/10.1186/s40816-019-0149-4.
- Oloyede, H.O.B., Lukman, H.Y. & Salawu, M.O. (2020). Protective potentials of ethyl acetate-ethanolic fraction of Carica papaya leaves against acetaminophen-induced liver damage in rats. *Notulae Scientia Biologicae*, *12*(3), 556-567. https://doi.org/10.15835/nsb12310629.
- Patil, K.R., Mahajan, U.B., Unger, B.S., Goyal, S.N., Belemkar, S., Surana, S.J., … Patil, C.R. (2019). Animal Models of Inflammation for Screening of Anti-inflammatory Drugs: Implications for the Discovery and Development of Phytopharmaceuticals. *International Journal of Molecular Sciences,* 20, 4367-4404. doi:10.3390/ijms20184367.
- Patil. K.R. & Patil, C.R. (2017). Anti-inflammatory activity of Bartogeni acid containing fraction of fruits of Barringtonia race mosa Roxb. In acute and chronic animal model of inflammation. Journal of *Traditional* and *Complementary Medicine*, 7, 86-93. https://doi.org/10.1016/j.jtcme.2016.02.001.
- Rhetso, T., Seshadri, R.M., Ramnath, S. & Venkataramegowda, S. (2021). GC-MS based metabolite profiling and antioxidant activity of solvent extracts of Allium chinense G Don leaves. *Notulae Scientia Biologicae,* 13, (2): 10791. DOI:10.15835/nsb13210791.
- Shinde, U.A., Phadke, A.S., Nari, M., Mungantiwar, A.A., Dikshit, V.J. & Sarsf, M.N. (1999). Membrane stabilization activity-A possible mechanism of action for anti-inflammatory activity of *Cedrusdeo-*

dora wood oil. *Fitoteropia,* 70, 251-257. PII: S 0 3 6 7 - 3 2 6 X 9 9 0 0 Ž .030-1.

- Sokeng, S.D., Rokeya, B., Hannan, J.M.A., Ali, L. & Kamtchouing, P. (2013). Antidiabetic and antiplatelet aggregation activities of *Brideliandellensis*stem bark extracts. *Journal* of *Diabetes Research*, 2, 13-19. Doi: 10.5923/j.diabetes.20130201.03.
- VasudhaUdupa, A., Gowda, B., Kumarswammy, B.E. & Shivanna, M.B. (2021). The antimicrobial and antioxidant property, GC–MS analysis of non-edible oil-seed cakes of neem, madhuca, and simarouba. *Bulletin of National Research Center*, 45, 41-54. https://doi.org/10.1186/s42269-021-00498-x.