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Research Article

Deciphering the therapeutic actions of *Brenania brieyi* (Rubiaceae) fractions on oxidoinflammatory anomalies

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Abstract: A decline in the antioxidant network during the inflammatory response plays a critical role in the pathogenesis of numerous diseases. We designed this study to decipher the therapeutic efficacy of *Brenania brieyi* in reducing oxidative stress caused by the inflammatory response to cotton pellets. Graded doses of methanol and chloroform fractions of *B. brieyi* (MFBB and CFBB) and indomethacin were administered to Wistar rats for seven days after implanting sterilised cotton pellets (20 mg). Thereafter, biochemical indices of oxidative stress were determined using blood samples taken through cardiac puncture. Furthermore, molecular interactions, drug-likeness, and toxicity features of *B. brieyi phytochemicals* were also assessed. Compared with the untreated group, the groups treated with MFBB and CFBB had a significant ($p < 0.05$) decrease in granuloma tissue weight and MDA levels while increasing glutathione levels, SOD, and CAT activities. In addition, a substantial increase in inflammatory-induced changes in antioxidant nutrients, together with a decline in liver enzymes, was obtained in the treated groups. The docking tests revealed that the top-scoring phytoconstituents of *B. brieyi,* n-hexadecanoic acid, and 9-octadecanoic acid interacted well with catalase, having docking scores of -6.19 and -7.58 kcal/mol, respectively. Moreover, the hits had good oral drug-likeness features and a safe toxicity profile. The findings of the study provide evidence that *B. brieyi* has antioxidant and anti-inflammatory properties, suggesting that it could be used as an alternative therapy to regulate oxidative stress-related diseases.

1. INTRODUCTION

The inflammatory cascade is essentially initiated by microbial agents, mechanical, chemicals, thermal stimulation, trauma, or autoimmune illnesses to defend the body from injury (Ashenafi *et al.*, 2023). Sadly, disproportionate or excessive inflammatory responses elicit an overwhelming increase in reactive oxygen species generation which skewed the redox equilibrium to an oxidative state (Belahcene *et al.*, 2024) Oxidative stress provokes oxidative

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modification of biomolecules (lipid, protein, and DNA) through lipid peroxidation, protein carbonylation, nitration, sulfoxidation, and carbonyl adduct formation (Alothaid, 2022; Chukwuma *et al*., 2023). Altogether, this interferes with redox signalling for cellular processes, resulting in defects in cell differentiation, proliferation, genomic stability loss, and biomolecule functionalities (Pisoschi *et al*., 2021).

Oxidative stress and inflammation are intertwined and intrinsically involved in the pathophysiology of several disease conditions, including cancer, diabetes, alcoholic liver diseases, chronic kidney disease, and cardiovascular and neurological diseases (Awan *et al*., 2023; Ezeorba *et al*., 2024) Emerging evidence from epidemiological and experimental studies has shown that the signal transduction pathway triggers inflammatory gene expressions, such as kinases, cytokines, and transcription factors, which mediate the interdependent relationship between the two (Pisoschi *et al*., 2021). Conversely, these processes act as a vicious cycle (Belahcene *et al*., 2024). For instance, in a disease state where inflammation is a primary event, oxidative stress usually develops as a consequence, which will further exacerbate inflammation and vice versa (Pisoschi *et al*., 2021). This potentiates the need to target both to subvert the onset and progression of chronic diseases since they work in concert. Although many orthodox drugs have been designed to address these pathologies, their adverse effects, including immune suppression, ulcers, gastrointestinal bleeding, and an increase in blood sugar and pressure, limit their usage (Chukwuma *et al*., 2021). As such, natural agents have recently been the subject of increased investigation as remedies for human diseases due to their great therapeutic value, affordability, and biocompatibility (Radi *et al*., 2023; Rodrigues *et al*., 2024). The medicinal plants' therapeutic potentials are hinged on the presence of various secondary metabolites (Nkwocha *et al*., 2022; Radi *et al*., 2023).

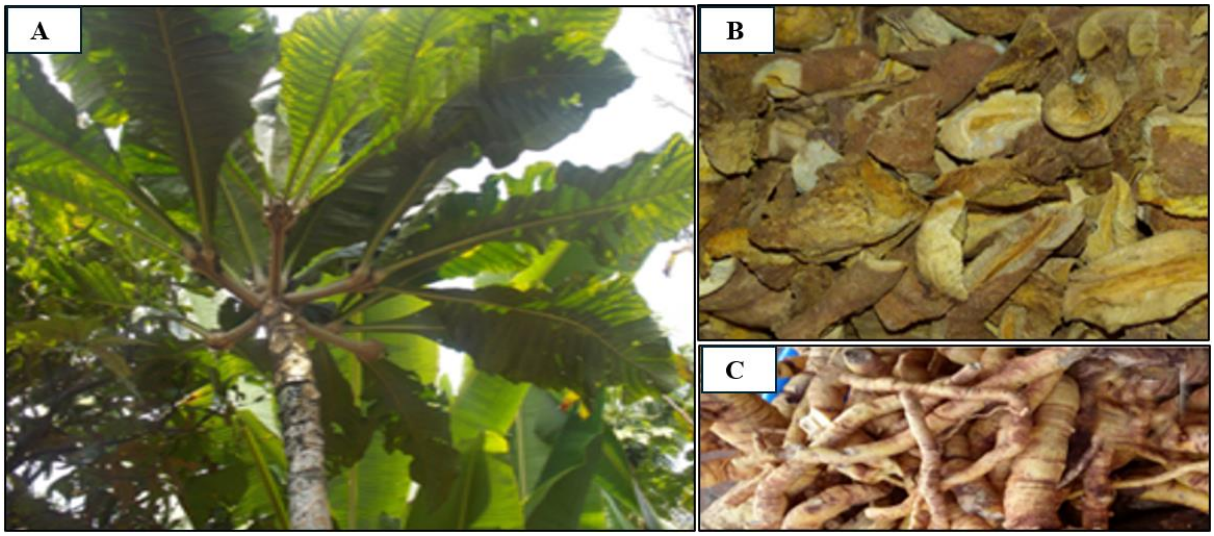

Figure 1. Pictorial view of *B. brieyi* plant (a), root (b) and root bark (c).

In the milieu of plants highly sought for inflammatory-mediated pathologies in ethnomedicinal practice is *Brenania briefyi* (De Wild.) E.M.A.Petit taxonomically classified under the Rubiaceae family of flowering plants. *B. brieyi* is predominantly distributed in Cameroon, Congo, Nigeria, Gabon, and the Central African Republic (Ezeala *et al*., 2023). The large leaves look similar to those of some species of the genus *Anthocleista* Afzel. ex R.Br. The leaves are rounded at apex while the stipules, which in most Rubiaceae help to identify the family, are small and fall early in this species. *B. brieyi* plant grows up to 30 m tall (Ezeala et al., 2023) (Figure 1). It has excellent value as an herbal therapy for swelling, pain, fever, and endocrine disorders. Previous studies identified pharmaceutical-relevant secondary metabolites such as phenols, flavonoids, saponins, terpenoids, alkaloids, and tannins in the root bark of *B. brieyi* (Chukwuma *et al*., 2022). Gas-chromatography characterization of the root bark also revealed the presence of 9-Ocadecanoic acid, hexadecanoic acid, octadecanoic acid, and

squalene, among others (Odo *et al*., 2017). These metabolites could be the basis for the plant's registered antipyretic, anti-inflammatory, analgesic, and *in vitro* antioxidant effects (Chukwuma *et al*., 2022). Given all the various applications of *B. brieyi* in the treatment of inflammatory-related diseases, its rich bioactive compounds, and the already established *in vitro* antioxidant activity of *B. brieyi* fractions, this study was designed to investigate the therapeutic efficacy of the fractions and their secondary metabolites against inflammation-mediated oxidative stress in Wistar rats.

2. MATERIAL and METHODS

2.1. Collection and Identification of Plant Materials

The roots bark of *B. brieyi* root used for this study was collected from Njikoka, Anambra State, Nigeria 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 the department's herbarium.

2.2. Preparation of the Fractions

The air-dried and grounded *B. brieyi* root bark (1793 g) was prepared at ambient temperature using methanol and chloroform (1:2 v/v) as extracting solvents for 48 h. The macerate was filtered using Whatman No. 1 filter paper, measured, and mixed with distilled water (20% volume). Then, the resulting solution was separated into two organic layers after vigorous shaking and separated using a separating funnel. The upper and lower layers were designated methanol fraction of *B. brieyi* root bark (MFBB) and chloroform fraction of *B. brieyi* root bark (CFBB), respectively, based on their molecular weight. The fractions were concentrated and kept in a refrigerator at 4 ºC.

2.3. Research Animals

The forty-five (45) Wistar rats of both sexes (average weight of 120.11 g; 8–12 weeks old) used for this investigation were housed in a cage at 25°C with a 12-hour dark/light cycle and given free access to rodent feed and water throughout the study. The rodents were randomized into nine groups (n =5) as follows:

Group 1: (No injection)
Group 2: Cotton pellet (20 mg) (not treated)
Group 3: Cotton pellet (20 mg) + indomethacin (10 mg/kg)
Group 4: Cotton pellet (20 mg) + MFBB (50 mg/kg)
Group 5: Cotton pellet (20 mg) + MFBB (100 mg/kg)
Group 6: Cotton pellet (20 mg) + MFBB (200 mg/kg)
Group 7: Cotton pellet (20 mg) + CFBB (50 mg/kg)
Group 8: Cotton pellet (20 mg) + CFBB (100 mg/kg)
Group 9: Cotton pellet (20 mg) + CFBB (200 mg/kg)

The protocol of Mosquera *et al*., (2011) was used to assess the effects of the fractions on cotton pellet-induced inflammation. The animals were treated for seven days and anaesthetized following the procedure of the American Veterinary Medical Association (AVMA) on the eighth day. Subsequently, their blood samples were collected in plain bottles and centrifuged, and the serum was used to determine biochemical markers of oxidative stress. Finally, the pellets surrounded by granuloma tissue were disserted and weighed after oven drying at 60 ºC.

2.4. Determination of Antioxidants and Biomarkers of Oxidative Stress

The status of antioxidants and oxidative stress biomarkers were measured using the following methods: Malondialdehyde levels (Wallin *et al*., 1993), activities of antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were assayed using, (Aebi, 1983), (Fridovich, 1989), and (Paglia & Valentine, 1967) methods, respectively. The non-enzymatic antioxidants were measured with these methods: Reduced

glutathione (GSH), (Beutler *et al*., 1963); total protein, (Tietz, 1995); total bilirubin and direct bilirubin, (Jendrassik & Grof, 1938); albumin, (Doumas *et al*., 1971); vitamin E, (Pearson, 1976); vitamin C, (Goodhart & Shils, 1973); zinc, (Johnsen & Eliasson, 1987); selenium, (Krishnaiah *et al*., 2003); iron (Teco diagnostic, USA). Experimental protocols of (Reitman & Frankel, 1957) were used to assay serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate activities.

2.5. Molecular Docking Studies

2.5.1. *Protein preparation*

The protein preparation wizard panel of Schrodinger Suite was used to prepare the crystallographic structure of catalase (PDB ID: 1DGB), which was obtained from the Protein Data Bank (PDB) in the following way: disulfide bonds were formed, bond orders were assigned, hydrogen atoms were added, side chains and loops that were missing were filled, and water molecules larger than 3.0 Å of heteroatoms were eliminated. OPLS2005 and PROPKA were used for the structure's minimization and optimization, respectively.

2.5.2. *Ligand preparation*

The compounds identified from *B. brieyi* (Odo *et al*., 2017) and ascorbic acid (a standard antioxidant) imported in the project table of Schrödinger Suite, 2020-3, from the PubChem database, were prepared for molecular docking via the Lipprep wizard panel.

2.5.3. *Protein-ligand docking*

Based on the site map analysis findings, the produced ligands were docked into the protein's optimal binding pocket using the glide docking tool of Schrödinger Maestro 12.5. A flexible ligand sampling method was used to get samples of all the set functional groups, ring conformations, and nitrogen inversions. The partial charge cut-off for ligand atoms was set at 0.15, and the vdW radius scaling factor was scaled at 0.80. Finally, the 2D and 3D interactions of ascorbic acid and the two top-scoring ligands (best docking scores) were examined.

2.6. ADMET Prediction

The ADME properties of compounds from *B. brieyi and* standard antioxidant (ascorbic acid) were obtained with canonical smiles of each compound using the Swiss ADME server, while the ProTox-II online server was used for toxicity screening.

2.7. Statistical Analysis

One-way analysis of variance helped to calculate the mean and standard deviation (SD) of the datasets obtained in the study. The differences between the means were measured at $P < 0.05$, at least significant level, using the Duncan post hoc multiple comparisons of SPSS Inc., Chicago, IL, USA.

3. RESULTS and DISCUSSION

3.1. Effects of MFBB and CFBB on Granuloma Tissue Weight

The cotton pellet inflammatory model is widely used to measure the efficacy of therapy against proliferative and exudative phases of chronic inflammation (Ashenafi *et al*., 2023). We found out that the weight of granuloma tissue formation was significantly lower ($p < 0.05$) in the groups that were given the fractions and the standard drug indomethacin compared to the group that was not treated (Figure 2). Interestingly, the group that was given 200 mg/kg of MFBB had the smallest granuloma tissue weight, which shows that it is very good at stopping chronic inflammation (Figure 2). Foreign agents, such as cotton pellets, incite foreign body granulomas, which incite macrophage, phagocytosis, and T cell-mediated immune responses (Chukwuma *et al*., 2021). It induces the infiltration of macrophages and inflammatory mediators into the inflamed region, which forms mast cells to wade off the foreign agent. Nevertheless, the majority of cells and mediators released may quicken the formation of ROS and RNS,

contributing to oxidative stress and more tissue damage (Kaufmanova *et al*., 2021). Therefore, the capacity of fractions to inhibit granuloma tissue formation suggests its effectiveness in suppressing the proliferative phase of the inflammation cascade, granuloma fibroblast formation, and oxidative stress after cotton pellet implantation, thereby making it a remedy for inflammation. This finding concurs with the reports of (Ashenafi *et al*., 2023), who also observed reduction in granuloma weight in rats treated with *Vernonia auriculifera* (Asteraceae) fractions.

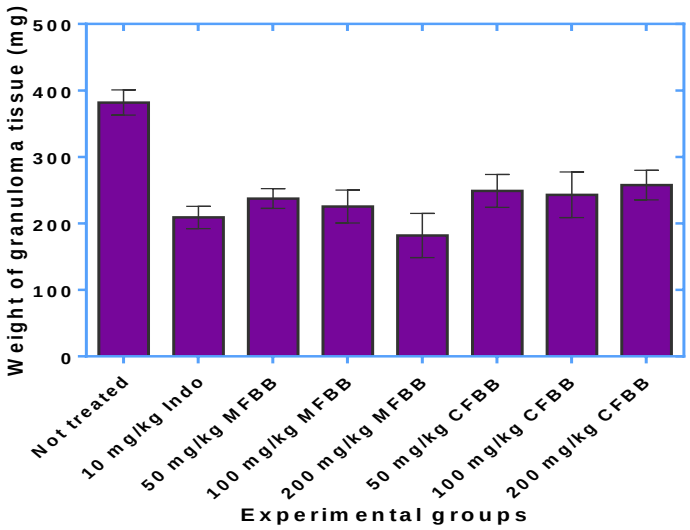

Figure 2. The weight of granuloma tissues (mg) formed after cotton pellet implantation.

3.2. Effect of MFBB and CFBB on Lipid Peroxidation and Antioxidant Enzymes Activities

Malondialdehyde is an oxidative stress biomarker used to quantify the extent of lipid peroxidation (Alothaid, 2022). Herein, a significant ($p < 0.05$) increase in the levels of MDA was recorded in rats not treated (group 2) compared to group 1 (no injection). However, a dose-dependent decrease in MDA levels was obtained in groups treated with 50, 100, and 200 mg/kg b.w. of MFBB and CFBB, as well as the standard drug (Figure 3). The fractions' bioactive compounds likely scavenged free radicals, stopping lipid peroxidation from starting, or they converted peroxy radicals to hydroperoxide before they could start a chain reaction, which triggers the propagation of radical chain reactions (Ezeorba *et al*., 2024). This finding is supported by the significant ($p < 0.05$) increase in the activities of SOD and CAT following treatment with the fractions relative to the untreated group. The drop in antioxidant enzymes in the untreated group could be because of a change in redox equilibrium, which causes these antioxidants to be over-utilized due to the escalated generation of oxidant species by inflammatory enzymes like leukotrienes and cyclooxygenase (Belahcene *et al*., 2024). Considering the functions of these antioxidants in the inactivation of reactive oxygen species, their restoration in the treated groups will subvert oxidative damage mediated by chronic inflammation. Thus, this suggests that the fractions might have served as scavengers of the free radical products of granular inflammation or improved the production of antioxidant enzymes, which shielded the cells from reactive substances, making them a target for stemming the health challenges associated with oxidative stress. This may be because of the phytochemicals—phenols, flavonoids, and tannins that were previously found in the fractions. These compounds have been shown to exhibit antioxidant activity through a variety of mechanisms, including metal ion chelation, scavenging of radicals, and stimulation of endogenous antioxidant expression (Chukwuma *et al*., 2023; Onyesife *et al*., 2023). It is worthy to note that other nonphenolic compounds are also implicated in the antioxidant actions of plant extracts (Gashaye & Birhan, 2023).

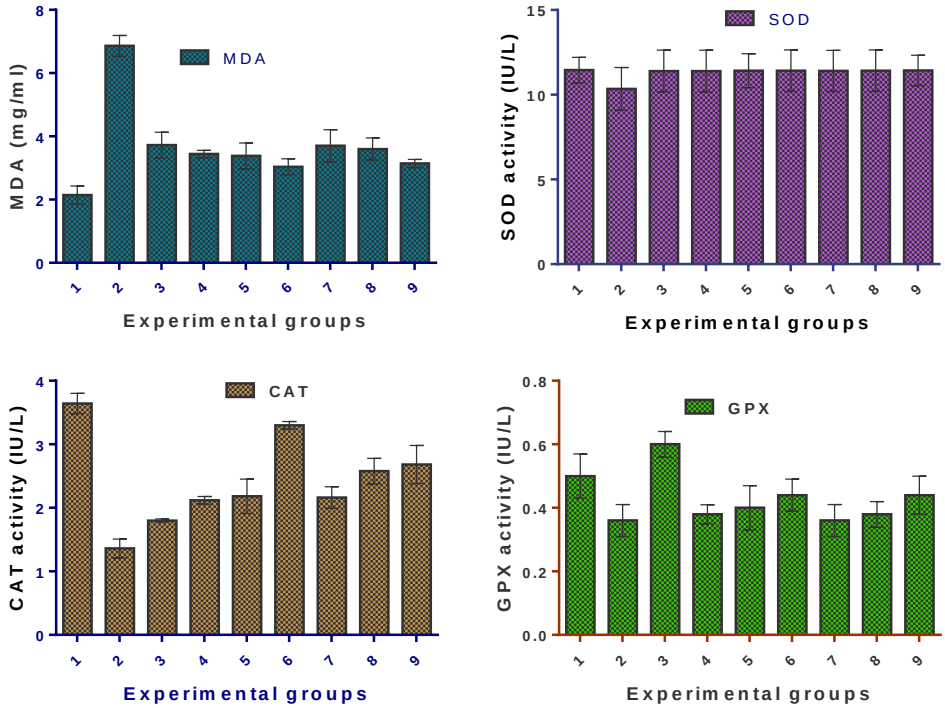

Figure 3. Effects of the MFBB and CFBB on serum MDA and antioxidant enzyme activities.
Group 1: no injection, Group 2: not treated, Group 3: Standard group, 10 mg/kg b.w indomethacin. Groups 4-6 received 50, 100, and 200 mg/kg b.w of MFBB, whereas Groups 7 -9 received 50, 100, and 200 mg/kg b.w of CFBB.

3.3. Effects of the MFBB and CFBB on Serum Metabolic Antioxidants

The results in Table 1 revealed that the cotton pellets elicited a decline in GSH, total protein, and albumin while increasing the levels of bilirubin in group 2 (not treated) compared with group 1 (no injection). The reduced levels of these endogenous antioxidants in the untreated group could be associated with the inflammatory-mediated generation of free radicals, which could impair immune response and susceptibility to infection (Hussen & Endalew, 2023). Notably, treatment with MFBB and CFBB attenuated these anomalies, with the effect being more pronounced in the group administered 200 mg/kg b.w. of MFBB (Table 1). Since studies have shown that glutathione is essential for defending cells against damage caused by free radicals, increasing glutathione levels by the fractions confers an additional therapeutic benefit in many inflammatory disorders (Alothaid, 2022). Similarly, the rise in total protein and albumin suggests that the fractions might have increased protein synthesis or reduced ROS-mediated protein modification. Elevation of bilirubin levels is a strong indication of hepatic injury due to impaired biliary function of the liver (Alothaid, 2022). Excess ROS can change the electrical charge of proteins, break peptide chains, cross-link proteins, and oxidise particular amino acids, creating protein adducts. It is worth mentioning that antioxidants inhibit bilirubin synthesis via inactivating heme oxygenase-1 (HO-1), an essential enzyme in bilirubin synthesis (Ryter, 2022).

Table 1. Effects of the MFBB and CFBB on Serum Metabolic Antioxidant Concentrations.

Groups	Glutathione (mg/ dL)	Total protein (g/ dL)	Direct bilirubin (mg/ dL)	Total bilirubin (mg/ dL)	Albumin (g/ dL)
1	2.62 ± 0.08^{f}	5.80± 0.45^{cd}	0.58 ± 0.04^{a}	1.38 ± 0.04^{a}	3.74 ± 0.11^{ab}
2	0.68 ± 0.08^{a}	3.86±0.24^{a}	1.78 ± 0.15^{e}	3.76 ± 0.17^{e}	3.48 ± 0.26^{a}
3	1.14 ± 0.18^{b}	5.18± 0.75^{bc}	0.94 ± 0.15^{b}	1.62 ± 0.084^{b}	4.06 ± 0.19^{bc}
4	2.24 ± 0.05^{e}	5.48 ± 0.43^{bcd}	1.46 ± 0.90^{d}	2.46 ± 0.15^{d}	4.26 ± 0.15^{c}
5	2.54 ± 0.13^{f}	5.56±0.68^{bcd}	0.84 ± 0.06^{b}	2.40 ± 0.07^{d}	4.06 ± 0.18^{bc}
6	2.58 ± 0.13^{f}	5.64±0.38^{bcd}	0.62 ± 0.13^{a}	1.92 ± 0.08^{c}	5.48 ± 0.43^{d}
7	1.58 ± 0.05^{c}	4.82±0.59^{b}	1.42 ± 0.04^{d}	2.04 ± 0.11^{c}	3.90 ± 0.43^{bc}
8	2.00 ± 0.12^{d}	5.28± 0.70^{bcd}	1.16 ± 0.11^{c}	1.96 ± 0.18^{c}	4.12 ± 0.19^{c}
9	2.22 ± 0.16^{e}	6.16±1.20^{d}	0.90 ± 0.10^{b}	1.72 ± 0.08^{b}	4.20 ± 0.10^{c}

Results are reported as mean ± standard deviation (n = 5*)*. The mean values in the same column containing different superscript alphabets indicate a significant difference ($p < 0.05$).
Group 1: no injection, Group 2: not treated, Group 3: Standard group, 10 mg/kg b.w indomethacin.Groups 4-6 received 50, 100, and 200 mg/kg b.w of MFBB, whereas Groups 7 -9 received 50, 100, and 200 mg/kg b.w of CFBB.

3.4. Effects of the MFBB and CFBB on Serum Nutrients Antioxidants

For optimum cellular activities, especially during situations of oxidative stress, nutrient antioxidants support endogenous antioxidants. Although group 2 (not treated) had a decrease in vitamins E and C, zinc, and selenium while increasing iron levels compared with group 1 (no injection), treatment with the fractions and indomethacin restored these anomalies (Table 2), with MFBB being more effective. Emerging evidence from previous studies has reported that vitamin C not only acts directly as a scavenger of ROS and RNS but also reduces both the tocophenol radical and the tocophenyl quinone. The generated tocopherol will, in turn, protect membrane lipids from lipid peroxidation induced by inflammatory reactions and peroxynitrite (Pisoschi *et al*., 2021). In the same vein, zinc helps to maintain the redox state by regulating transduction pathways, activator genes, as well as the antioxidant enzymes. In addition, selenium is an integral part of selenoproteins and some antioxidant enzymes, which enhance vitamin C regeneration, Cu^{+} chelation, and protect the cells against free radicals and DNA damage (Pisoschi *et al*., 2021). Reducing iron levels after treatment with the fractions is vital for hemostasis since excess iron can cause metabolic dysfunction and severely threaten cell survival in oxidative environments. Conversely, restoration of these antioxidant nutrients will help to restore redox equilibrium and cellular homeostasis under an inflammatory response, which is the basis for preventing metabolic diseases (Ashenafi *et al*., 2023; Awan *et al*., 2023).

Table 2. Effects of the MFBB and CFBB on Serum Nutrient Antioxidants.

Groups	Vitamin C (mg/dL)	Vitamin E (mg/dL)	Iron (µg/dL)	Zinc (µg/dL)	Selenium (µg/dL)
1	1.42 ± 0.10^{d}	0.62 ± 0.02^{cd}	107.46 ± 7.18^{a}	170.36 ± 7.53^{d}	2.86 ± 0.11^{d}
2	1.02 ± 0.04^{a}	0.42 ± 0.03^{a}	210.44 ± 8.96^{e}	134.72 ± 7.15^{a}	1.72 ± 0.33^{a}
3	1.06 ± 0.05^{a}	0.56 ± 0.04^{abcd}	120.14 ± 2.60^{b}	159.62 ± 8.37^{cd}	2.08 ± 0.04^{bc}
4	1.08 ± 0.08^{ab}	0.46 ± 0.07^{ab}	206.72 ± 4.27^{e}	152.54 ± 5.60^{bc}	2.02 ± 0.04^{bc}
5	1.12 ± 0.04^{ab}	0.52 ± 0.03^{abc}	157.46 ± 7.15^{d}	194.00 ± 11.25^{e}	2.08 ± 0.04^{bc}
6	1.22 ± 0.08^{c}	0.70 ± 0.04^{d}	160.44 ± 5.90^{d}	198.88 ± 5.19^{e}	2.22 ± 0.04^{c}
7	1.04 ± 0.05^{a}	0.56 ± 0.05^{abcd}	162.68 ± 7.72^{d}	144.40 ± 9.17^{ab}	2.10 ± 0.10^{bc}
8	1.04 ± 0.11^{a}	0.60 ± 0.05^{bcd}	138.14± 6.43^{c}	190.94 ± 6.80^{e}	1.96 ± 0.11^{b}
9	1.18 ± 0.04^{bc}	0.56 ± 0.02^{abcd}	126.32 ± 10.05^{b}	192.90 ± 13.76^{e}	2.16 ± 0.18^{bc}

Results are reported as mean ± standard deviation (n = 5*)*. The mean values in the same column containing different superscript alphabets indicate a significant difference ($p < 0.05$).
Group 1: no injection; Group 2: not treated, Group 3: Standard group, 10 mg/kg b.w indomethacin.Groups 4-6 received 50, 100, and 200 mg/kg b.w of MFBB, whereas groups 7 -9 received 50, 100, and 200 mg/kg b.w of CFBB.

3.5. Effects of the MFBB and CFBB on Serum Liver Enzymes Activities

The liver function panel measures the activities of AST, ALT, and ALP, which are significant and sensitive markers of hepatic assault (Alothaid, 2022). Hence, we assessed the activities of liver enzymes (AST, ALT, and ALP) to ascertain the hepatocellular status of the rats after chronic inflammation. Our results showed a significant ($p < 0.05$) elevation in AST, ALT, and ALP activities in the untreated group relative to baseline (Table 3). Accordingly, the elevation of these liver enzymes in the untreated group suggests liver injury and an alteration in membrane permeability, which released these enzymes into the blood. This is possible since there was also an increase in MDA, a commonly used indicator of lipid peroxidation, in the untreated rats (Alothaid, 2022). Interestingly, indomethacin (group 3), MFBB, and CFBB-treated groups (5, 6, 8, and 9) registered a decline in AST, ALT, and ALP activities (Table 3). The decrease in the liver enzymes assayed in the treated groups could be hinged on the phytochemicals present in the fractions. Studies have shown that phenols and flavonoids affluent in the fractions exert antioxidant and hepatic protective effects since they prevent membrane hemolysis, lipid peroxidation, and, ultimately, leakage of liver enzymes into the blood.

Table 3. Effects of the MFBB and CFBB on Serum Liver Enzymes Activities.

Groups	Treatments	Doses (mg/kg b w)	AST (u /l)	ALP (u/l)	ALT (u/l)
1	Baseline	-	39.40 ± 3.21^{e}	64.28 ± 2.32^{a}	22.00 ± 4.18^{a}
2	Control		67.00 ± 2.23^{f}	124.64 ± 19.96^{e}	76.00 ± 6.89^{e}
3	Indomethacin	10	57.00 ± 2.54^{cd}	96.18 ± 12.15^{cd}	51.00 ± 6.12^{c}
4	MFBB	50	64.00 ± 7.03^{ef}	93.40 ± 9.84^{bcd}	71.80 ± 6.87^{de}
5		100	52.20 ± 4.49^{bc}	81.20 ± 6.54^{bc}	69.60 ± 1.14^{de}
6		200	50.40 ± 3.65^{b}	72.66 ± 16.50^{b}	65.40 ± 5.31^{d}
7	CFBB	50	61.00 ± 3.39^{def}	123.76 ± 17.21^{e}	69.20 ± 6.41^{de}
8		100	59.60 ± 5.98^{de}	102.28 ± 17.73^{d}	45.60 ± 3.71^{c}
9		200	52.60 ± 5.50^{bc}	74.48 ± 20.67^{b}	31.60 ± 3.85^{b}

Results are reported as the mean ± standard deviation (n = 5). The mean values in the same column containing different superscript alphabets indicate a significant difference ($p < 0.05$)

3.6. Molecular Interaction of *B. brieyi* Phytochemicals with Catalase

Molecular docking studies show that the binding affinity of - 6.19 and 7.58 kcal/mol was obtained for hexadecenoic and 9-octadecanoic acids, respectively, which is higher than the -6.07 kcal/mol obtained for ascorbic acids (Figure 4). The docking studies established two hydrogen bonds in both compounds with positively charged HP 362 and ARG 365 amino acid residues using carbonyl groups attached to their structures (Figure 4). Similarly, ascorbic acids formed two hydrogen bonds between positively charged ARG 72 and GLY 147 using carbonyl and hydroxyl groups attached to the straight chain (Figure 4). According to (Apeh *et al*., 2023), enzyme catalysis and the structural stability of biomolecules both depend on hydrogen bonding with ligands. Interestingly, the report of this study is in agreement with previous studies by (Sierra-campos *et al*., 2020) who also reported the interaction of *Moringa oleifera* (Moringaceae) with catalase. The distance between the random structure and the α-helix structure increases through this contact, which in turn boosts catalase's activity (Sierra-campos *et al*., 2020).

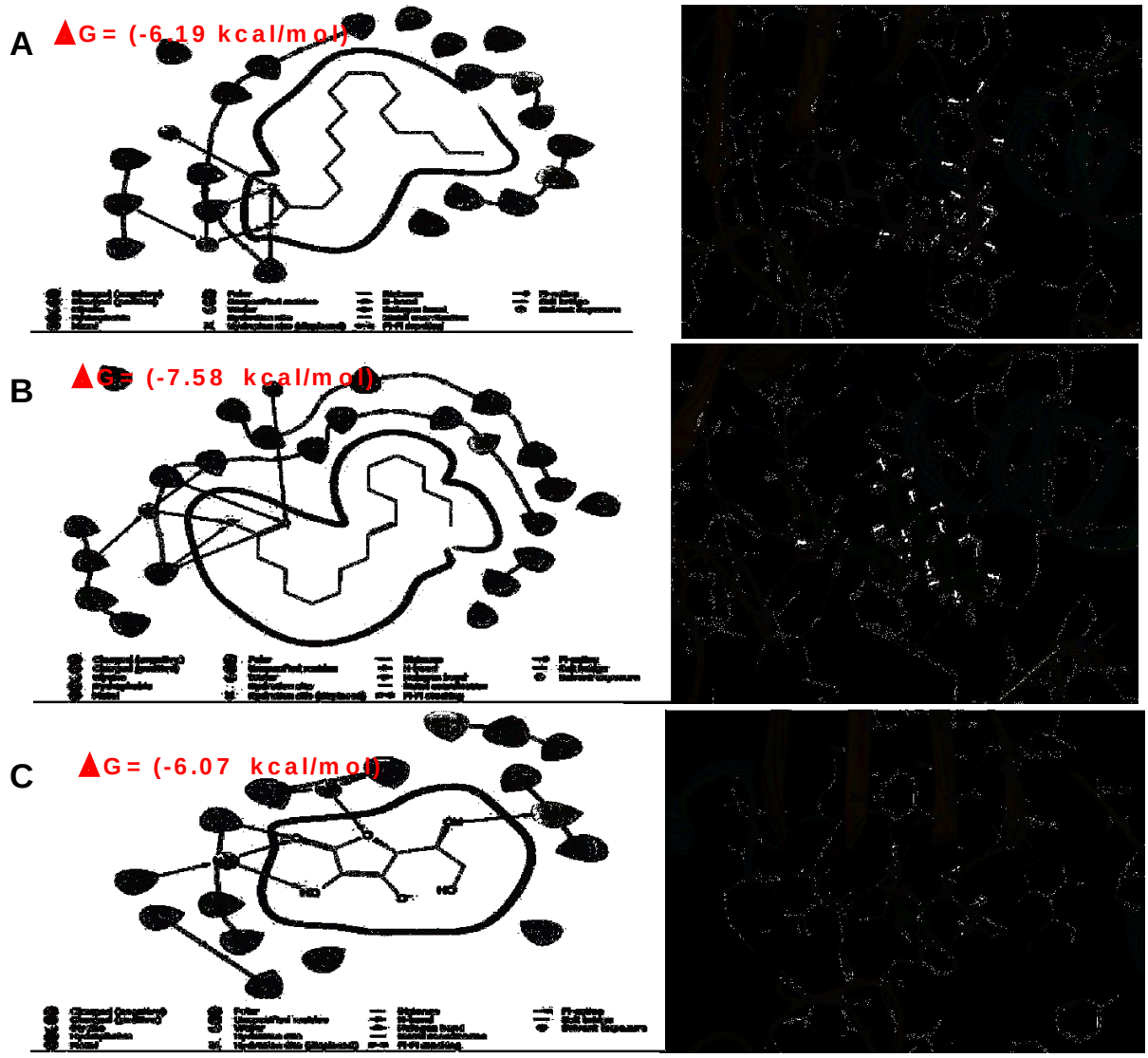

Figure 4. Molecular interaction of hexadecenoic (A), 9-octadecanoic acids (B) and ascorbic acid (C) with catalase. 2D left; 3D right.

3.7. ADMET Prediction

The ADME prediction for oral drugs is crucial in evaluating the drug-likeness and pharmacokinetics of oral medications (Yusuf *et al*., 2023). Hexadecenoic and 9-octadecanoic acids, just like the standard antioxidants (ascorbic acid) and anti-inflammatory (prednisolone, and indomethacin) drugs, passed the Lipinski rule of drug-likeness based on their < 500 molecular weight, < 5 iLog P, < 5 hydrogen bond donor and < 5 hydrogen bond acceptor (Apeh *et al*., 2021). Besides, the toxicity profile shows that the LD_{50} of hexadecenoic and 9-octadecanoic acids were 900 and 1925, respectively, making them safe within these dosage ranges. They were also safe based on their carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity profiles, except for 9-octadecanoic acid, which is hepatotoxic. However, the high LD_{50} values indicate that consumption of lower doses will be safe, thereby averting hepatoxicity. Hence, they are safer than indomethacin with low LD_{50} values (12 mg/kg), belong to the high toxicity class (class 2), and are also hepatotoxic and immunotoxic (Table 4).

Table 4. ADMET Prediction of *B. brieyi* compounds and standard drugs.

	A	B	C	D	E
Drug likeness					
Molecular weight ≤ 500	256.42	282.46	176.12	360.44	357.99
Hydrogen bond donor ≤ 5	1	1	4	3	1
Hydrogen bond acceptor ≤ 5	2	2	6	5	4
Octanal water partition coefficient					
Solubility class	Moderately soluble	Moderately soluble	Highly soluble	soluble	Moderately soluble
Mean log P	3.85	4.16	0.39	2.19	2.76
ESOL log S	-5.02	-5.7	0.23	-2.96	-4.86
Lipinski violation	1	1	0	0	0
Toxicity profile					
LD_{50} (mg/kg)	900	1925	3367	1680	12
Toxicity class	4	4		4	2
Hepatotoxicity	-	+	-	-	+
Carcinogenicity	-	-	-	-	-
Immunotoxicity	-	-	-	+	+
Mutagenicity	-	-	-	-	-
Cytotoxicity	-	-	-	-	-

Active = (+), Inactive = (-), A = n-hexadecanoic acid, B = 9-octadecanoic acid, C = Ascorbic acid, D = Prednisoline and E = indomethacin.

4. CONCLUSION

The findings from this research study disclosed that *B. brieyi* fractions have astonishing antioxidant and anti-inflammatory activities. The fractions inhibited granuloma tissue formation and lipid peroxidation and also restored the inflamed rats' endogenous and exogenous antioxidant status. Collectively, both the experimental-based and molecular studies validated the antioxidant actions of the fractions. This implies that it might be used as a therapeutic agent for oxidative stress pathologies.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s). The study was approved by the Faculty of Biological Sciences Ethics and Biosafety Committee, University of Nigeria. **Ethics Committee Number:** UNN/FBS/EC/1049.

Authorship Contribution Statement

IFC: Conceptualization. **IFC, LUSE** and **VNO**: Study design. **IFC, VOA, NFN, AEA** and **MOO**: Investigation. **VOA** and **AEA**: Analysis and docking, **IFC, VOA, NFN, AEA**, and **MOO**: Writing - original draft. **LUSE** and **VNO**: Writing - review and editing. All authors read and approved the final manuscript before submission.

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