Morphological, Biochemical and Molecular Docking Evaluation of the Anti-inflammatory Effects of Methanolic Extract of Bridelia ferruginea stem bark on Acetic acid-induced Ulcerative Colitis in Rats

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Summary: Ulcerative colitis (UC) is a chronic disorder that involves inflammation. This study was carried out to examine the anti-inflammatory effect of the methanol extract of Bridelia ferruginea stem bark in acetic acid-induced ulcerative colitis in male Wistar rats. Twenty-four rats were randomly divided into 6 groups of 4 animals each, colitis was thereafter induced by intrarectal administration of 4% (v/v) acetic acid in all except group 1, which received distilled water. For post-colitis induction treatment group 2 received distilled water, groups 3, 4 and 5 were orally administered the extract at doses of 100mg/kg, 200mg/kg and 400mg/kg, respectively while group 6 received sulfasalazine 500mg/kg orally. Post colitis induction, treatment lasted for 7 days and at the end of the experiment, colon samples were collected for estimation of antioxidant, inflammatory and histological parameters. Molecular docking study was also carried out to gain more insights about the promising anti-inflammatory compounds earlier identified in the extract. Results revealed that the extract significantly (p<0.05) attenuated the increased MDA, nitrite, TNF-α and IL-6 levels. Activities of SOD, CAT, MPO and GSH levels were also, significantly (p<0.05) increased. Furthermore, molecular docking study revealed that α-amyrin may have contributed significantly to the anti-inflammatory activity of the extract because of its remarkable binding affinity for IL-6, iNOS, IL1-β, TNF-α and COX-2 relative to prednisolone and celecoxib. This study suggests that the extract attenuated acetic acid-induced colitis via antioxidative and anti-inflammatory mechanisms.

Keywords: acid, ulcerative colitis, Bridelia ferruginea, cytokines, molecular docking

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INTRODUCTION

Ulcerative colitis (UC) is a chronic condition that causes inflammation and ulcers of the colon and rectum (Gisbert and Chaparro, 2019). It is an inflammatory bowel disease (IBD) with main symptoms being abdominal pain and diarrhea mixed with blood accompanied with weight loss, fever and anemia. Symptoms often appear gradually from mild to severe intermittent periods of no symptoms between flares. Complications may include abnormal dilation of the colon (megacolon), inflammation of the eye, joints, liver and colitis associated colon cancer (Wanderas et al., 2016; Greuter and Vavricka, 2019).

The manifestation of IBD globally was 59.25 per 100 000 people in 2019 (Wang et al., 2023). These figures are usually higher in western countries than other places in Africa (Alegbeleye, 2019; Hodges and Kelly, 2020). However, adoption of a western lifestyle (Zinocker and Lindseth, 2018; Hodges and Kelly, 2020) by Africans including Nigerians has increased the number of reported cases (Hodges and Kelly, 2020).

The etiology of the disease is unknown but some factors including genetics, environmental, microbial and immunological have been implicated in its pathogenesis, such that excessive immune responses initiate release of neutrophil infiltration, cytokines and other mediators which can cause damage to the colon tissues (Tian et al., 2017; Gupta et al., 2022).

Medications such as the 5-amino salicylic acid (5-ASA) derivatives, steroids, antibodies, anti-tumor necrosis factor (TNF)-alpha and other agents that can crush immune responses have been helpful in the treatment of IBD (Lamb et al., 2019; Hernandez-Rocha and Vande, 2020) but they do not come without side effects in addition to the fact that some individuals do not respond to treatment with them (Papamichael and Cheifetz, 2019). Currently, there are no other therapeutic agents available to effectively suppress immune response, inflammation and oxidative stress in all individual patient (Cai et al., 2021) and as such, it is...
important to identify a better, yet effective agent with fewer and milder side effects in the management of ulcerative colitis.

*Bridelia ferruginea* (BF) is a plant of the family-Euphorbiaceae, genus-*Bridelia*, commonly found in Savannah regions. The Folkloric use of decoction from its various part in Africa includes purgative and vermifuge. Several studies have reported the anti-inflammatory properties and activities of *Bridelia ferruginea* stem bark (Akuodor et al., 2012; Oloyede et al., 2014). Akuodor et al., (2011) reported the effects aqueous extract of BF on pain. Adetutu et al., (2011) also reported the antibacterial, antioxidant and healing properties of BF. In spite of the several ethnopharmacological reports on *Bridelia ferruginea*, its effects on colon inflammation are not known, hence the effect of its stem bark methanolic extract on acetic acid-induced colitis was investigated in male Wistar rats.

**MATERIALS AND METHODS**

**Drugs and chemicals:** acid (Qualikems, India), ketamine hydrochloride (Ciron Drugs & Pharmaceuticals, India), trichloro acid (TCA, Sigma, Germany), thiobarbituric acid (TBA, Sigma, Germany), 5, 50 dithio-bis-2-nitrobenzoic acid (DTNB, Sigma, Germany), hexadecyltrimethyl ammonium bromide (HTAB, Sigma, Germany) and Adrenaline (Sigma, Germany). Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were carried out by ELISA kits from Biologem, USA.

**Plant extraction:** The plant extract was harvested at Oke-atu Abeokuta North, Abeokuta, Ogun state. Fresh bark peels of the plant were air-dried, pulverized and extracted exhaustively in methanol. The filtrate was concentrated and evaporated to dryness at 60°C with rotary evaporator (Stuart Barloworld, Model RE 300) and stored in a refrigerator until use for the experiments.

**Animals:** Male Wistar rats weighing 150 – 200 g were purchased from Mctemmy concepts laboratory animal for research. They were kept in cages and aclimatized for twenty-one days under normal environmental condition before the study commenced. The rats were allowed food and water freely. The norm of Laboratory Animal Care was strictly complied with throughout the experiment (Garber et al., 2011).

**Experimental design:** Rats were divided into 6 groups as follows; Control group received no treatment throughout this study. Vehicle group, groups 3, 4, 5 and 6 received 1 ml of 4% (v/v) acetic acid through the rectum once for the generation of colitis (Fabia et al., 1992) (n = 5 animals per group). Groups 3, 4 and 5 were subsequently administered BME at 100, 200, and 400 mg kg⁻¹ respectively, per oral (p.o) for 7 days while group 6 was administered with sulfasalazine at 500 mg kg⁻¹ (p.o) for 7 days. At the termination of the study, rats were euthanized with ketamine anesthetics (10 mg per kg intraperitonially). Colonos from all rats were excised for antioxidant assays and histologic assessment.

**Biochemical assessments of inflammation:** Excised colon sections of rats were homogenized in sodium phosphate buffer (pH 7.4, 0.1M) and centrifuged at 4°C at a speed of 1 x 10⁴ rpm for 6 x 10² secs to obtain the supernatants which were thereafter stored at -20°C.

**Estimation of malondialdehyde (MDA) concentrations in colon tissue homogenates:** The MDA, an index of lipid peroxidation was estimated by the method of assay of thiobarbituric reacting substances (TBARS) (Nagababu et al., 2010). In brief, 0.1mL of supernatant was diluted 20 times in 1.5x10⁻³ M Tris-KCl buffer, and deproteinized with 0.5mL trichloro acid (30%). The mixture was subsequently centrifuged at 4 x 10⁴ rpm for 6x10² secs at room temperature. 0.2 mL of the supernatant was separated into Eppendorf tube, the 0.2 mL thiobarbituric acid (0.75%) was subsequently added and the combination was heated at 80°C for 60 mins. The tubes were chilled with ice pack, then 0.2 mL of the cooled mixture was removed into a microplate reader (Labtech LT-4500, UK) and absorbance was measured at 532 nm. The result was calculated using an index of absorption for MDA (molar extinction coefficient 1.56 x 10⁵/M/cm) and the concentration of TBARS in tissues were expressed as μmol MDA/mg protein.

**Estimation of nitrite concentrations in colon tissue homogenates:** Nitrite, as an indicator of nitric oxide (NO) production was measured according the method described by (Green et al., 1982). Briefly, 0.1mL of the supernatant was added to a microtiter plate and incubated with 0.1mL freshly prepared Griess reagent for ten minutes at room temperature in the dark. Sodium nitrite (0-100 μM) was formulated as standard to obtain the standard curve. The absorbance was estimated at 540 nm in a microplate reader (Labtech LT-4500, UK). The concentration of nitrite was determined from sodium nitrite standard curve and expressed as μM nitrite/mg protein.

**Estimation of glutathione (GSH) concentrations in colon tissue homogenates:** Reduced glutathione (GSH) was estimated in the tissue supernatant (Jollow et al., 1974). Briefly, 0.1mL of supernatant was diluted 20 times in 1.5 x 10⁻² M Tris-KCl buffer, and deproteinized with 0.5 mL trichloro acid (30%). The combination was centrifuged at 4 x 10⁴ rpm for 6 x 10² secs at room temperature. The 0.1 mL of the deproteinized supernatant was mixed with 0.1mL of 5¹, 5¹-Dithios-nitrobenzoic acid (DTNB, 0.0006 M) in a microplate plate. The absorbance was read within five min at 405 nm in a microplate reader (Labtech LT-4500, UK). The glutathione concentration was deduced from standard curve of glutathione (0-200 μM) and expressed as a μM GSH/mg protein.

**Assay of myeloperoxidase (MPO) activity in colon tissue homogenates:** Myeloperoxidase was extracted from homogenized tissue by suspending the material in 0.5% hexadecyl trimethyl ammonium bromide (HTAB) in 50 mM potassium phosphate buffer, pH 6.0, before sonication in an ice bath for 10 seconds. The specimens were freeze-thawed 3 times, after which sonication was repeated. Suspensions were then centrifuged at 4 x 10⁴ rpm for 15 min and the resulting supernatant was assayed spectrophotometrically for MPO. 0.1 ml of the supernatant was mixed with 290 uL of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.0005% H₂O₂.
The change in absorbance at 460 nm was estimated in a microplate reader (Labtech LT-4500, UK). One unit of MPO activity was defined as that degrading one micromole of H₂O₂ per minute at 25°C (U/mg protein).

Assay of catalase activity in colon tissue homogenates: It was determined using the colorimetric assay founded on a complex formed with molybdate and H₂O₂ (Goth, 1991). In brief, 0.1mL diluted supernatant of the colon homogenates was introduced into a microplate reader, and thereafter 0.05 mL of the combination of H₂O₂ (65 mmol/mL) and Na⁺–K⁺ phosphate buffer (60 mM, pH 7.4) was added. The consequent reaction was incubated for 3 min and discontinued with 0.1 mL of ammonium molybdate (64.8 mM) in sulfuric acid. The absorbance at 405 nm was estimated in a microplate reader (Labtech LT-4500, UK). The enzyme activity unit was presented as U/mg protein.

Assay of superoxide dismutase (SOD) activity in colon tissue homogenates: The level of SOD activity was decided by the approach of Misra and Fridovich (Misra & Fridovich, 1972). Superoxide dismutase activity is established on its capability to prevent the autoxidation of adrenaline in sodium carbonate buffer (pH 10.7). 50 µL of supernatant diluted twice was introduced into a microtitre plate holding 0.15mL of carbonate buffer. Thereafter, 0.03mL of freshly prepared 0.3 mM adrenaline was added to the mixture and the reaction. Blank was prepared using 0.05mL of distilled water. The rise in absorbance at 495 nm was observed every 60 seconds in four minutes. Activity was presented as U/mg protein.

Determination of tumor necrosis factor alpha (TNF-α) and Interleukin 6 (IL-6) levels in colon tissue homogenate: This was determined with the use of Biolegend ELISA kit, (USA) exclusive to the TNF-α and IL-6 with sensitivity constraint of 4 pg/mL with the use of microplate reader of 450nm filter. Biolegend instructions were followed through in all the measurements at room temperature. The concentration of the cytokines was extrapolated from the curves of IL-6 and TNF-α standards contained in the assay kits and subsequently expressed as pg/mL.

Histologic assessment of the colon: Samples of the colon tissue were processed for colitis estimation by an investigator shaded to study design and analysis was described (Owen et al., 2011). In brief, the tissues were fixed in formalin (10%). Water was removed in graded alcohol. Thereafter, it was cleared in xylene and fixed in formalin (10%). Water was removed in graded alcohol. Thereafter, it was cleared in xylene and fixed in paraffin wax. The tissues were later cut into sections (four micrometer thick) by a microtome, embedded on the slides and stained with hematoxylin and eosin (H&E). The resultant slides were examined underneath a light microscope (Olympus, Japan) and photomicrographs were taken with DM750 camera (Leica, Germany) at 100 magnifications.

Statistical Analysis: Results were presented as mean ± SEM. Data were compared for significant main effect using analysis of variance(one-way) and followed by Newman–Keuls post-hoc multiple-comparison test (unless otherwise stated). Data analysis and graph plots were performed with GraphPad 5.0 software (GraphPad Software Inc). Values were considered statistically significant when p < 0.05.

Protein preparation: The crystal structures of IL-6 (PDB ID of 1ALU), TNF-α (PDB ID of 2AZS), iNOS (PDB ID of 1r35) IL1B (PDB ID of 9ILB) COX 2 (PDB ID of 3LN1) were recalled from the protein data records (http://www.rcsb.org). The protein structures were prepared by removing bound ligands and water molecules while the required hydrogen atoms were added by using Discovery Studio, 2021. Using PyRx 0.8, the proteins were converted to Protein Data Bank, Partial Charge, and Atom Type (PDBQT) format to be used for molecular docking.

Ligand preparation: The structure data file (SDF) format of prednisolone, celecoxib, and the seventeen most abundant compounds identified in B. ferruginea methanolic extract in earlier study (Omoloso et al., 2021), were retrieved from http://www.pubchem.ncbi.nlm.nih.gov, (PubChem database). Open Babel built into PyRx 0.8 was operated to convert the ligands into PDBQT format to be used for molecular docking, using Vina (Trott & Olson, 2010)

Molecular Docking: Vina (Trott & Olson, 2010) incorporated into PyRx 0.8 was employed for docking and binding affinity determination. The individual enzyme in PDB format were brought in into the PyRx and changed to PDBQT, while the ligands were imported through Open babel(O’Boyle et al., 2011)where energy minimization before conversion to PDBQT format. A cluster analysis based on RMSD values, with reference to the starting geometry was subsequently executed and the minimum energy conformation of the most populated cluster was taken as the most reliable solution. The binding energy of the ligands for the five targets were documented and then ranked by their affinity values. Molecular interactions between the outstanding compound and the protein targets were viewed with the aid of Discovery Studio Visualizer, BIOVIA, 2021.

RESULTS

Bridelia ferruginea methanolic extract (BFME) improves macroscopic signs of experimental colitis: Control rat’s colon appeared normal macroscopically, intra-rectal administration of acetic acid in the vehicle treated rats however, caused colonic inflammation evidenced by hyperemia, edema and ulcers. On the contrary, rats administered with BFME at 100, 200, and 400 mg kg⁻¹ showed mild edema, colonic inflammation, thickening and ulceration while sulfasalazine treated rats showed similar macroscopic appearance as seen in rats given BFME with moderate colonic inflammation, edema, ulceration and less thickening (Figure 1).

Bridelia ferruginea methanolic extract (BFME) reduced Malondialdehyde (MDA) levels during experimental colitis in rats: The mean values of MDA levels in rat’s colon tissue homogenate post intrarectal administration of acetic acid are presented on Figure 1.
The generation of colitis with acetic acid was followed by significant rise in mean MDA level to 15.91 ± 0.29 nmol/mg protein in vehicle group compared to the control group which was 6.75 ± 0.74 nmol/mg protein. The MDA level decreased significantly (p < 0.05) in rats treated with BfME at 100, 200, and 400 mg kg⁻¹; mean values are 12.42 ± 0.62, 10.70 ± 0.71, and 10.06 ± 0.67 nmol/mg protein respectively, when compared with rats in the vehicle group. Sulfasalazine also, decreased mean MDA level to 10.52 ± 0.33 nmol/mg protein compared to Vehicle.

Bridelia ferruginea methanol extract (BfME) reduced nitrite levels during experimental colitis: The mean values of nitrite level in rat’s colon tissue homogenate post intrarectal administration of acetic acid are presented on Fig. 2. Colitis induction with acetic acid was attended with significant rise in nitrite levels. The value of Nitrite level in tissue homogenate was significantly (p < 0.05) raised in the Vehicle treated rats (mean value 5.34 ± 0.44 nmol/mg protein) when compared with values obtained in the control group (mean value 2.38 ± 0.14 nmol/mg). BfME treatment at 100, 200, and 400 mg kg⁻¹ prevented the increase in the Nitrite level with mean values being 3.26 ± 0.33, 2.68 ± 0.24, and 2.58 ± 0.15 nmol mg⁻¹ protein, respectively, matched with values in vehicle treated animals. Sulfasalazine significantly reduced mean of the level of nitrite to 2.48 ± 0.17 nmol/mg protein when compared to Vehicle.

**Figure 2:** Quantification of nitric oxide (NO) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

**Figure 3:** Quantification of myeloperoxidase (MPO) activity during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

(*Anti-inflammatory effects of Bridelia ferruginea on acid-induced Ulcerative Colitis*)
**Bridelia ferruginea** methanol extract (BIME) reduced myeloperoxidase (MPO) activity during experimental colitis: The mean values of MPO activity in rat’s colon tissue homogenate post intrarectal administration of acetic acid are presented on Fig. 3. Mean MPO activity was raised significantly to 30.83 ± 0.58 U/mg protein in the Vehicle group when matched with values in the Control group which had mean MPO activity values as 11.27±0.83U/mg protein. BIME at 100, 200, and 400 mg kg⁻¹ significantly attenuated MPO activity in that it reduced MPO activity values to 24.29 ± 1.37, 23.16 ± 1.84, and 18.28 ± 0.75 U/mg protein respectively, compared to vehicle. Sulfasalazine significantly decreased mean MPO activity to 10.04 ± 1.21 U/mg protein compared to Vehicle.

**Bridelia ferruginea** methanol extract (BIME) improved reduced glutathione (GSH) concentration during experimental colitis: The mean values of GSH concentration in rat’s colon tissue homogenate after administration of acid via the rectum are presented on Fig. 4. Intrarectal administration of acid in the Vehicle resulted in significantly decreased GSH concentration; 1.95 ± 0.15 nmol/mg protein compared with Control which was 4.15±0.42nmol/mg protein. Mean GSH concentration post intrarectal administration of acid in rats administered with BIME at a dosage of 100 mg kg⁻¹ was 2.39 ± 0.23, and in rats administered with BIME at dosages of 200 and 400 mg kg⁻¹, GSH concentration significantly increased to 5.98 ± 0.55 and 6.64 ± 0.78 nmol/mg protein respectively, compared to Vehicle. Sulfasalazine significantly increased mean GSH concentration to 10.13 ± 0.20 nmol/mg protein compared to Vehicle.

**Bridelia ferruginea** methanol extract (BIME) improved Superoxide dismutase (SOD) activity during experimental colitis: The mean values of SOD activity in rat’s colon tissue homogenate following intrarectal administration of acetic acid is presented on Figs. 5. Colitis induction was followed with significant reduction of superoxide dismutase (SOD) enzyme activity in control group; mean value being 0.39±0.03U/mg¹ protein. acid administered via the rectum significantly reduced the mean activity level of SOD to 0.21 ± 0.01 U/mg¹ protein when matched with values observed in the control group. BIME significantly increased mean SOD activity to 0.34 ± 0.01, 0.34 ± 0.02, and 0.31 ± 0.01 U/mg protein at the dosages of 100, 200, and 400 mg/kg, compared to vehicle. Sulfasalazine also, significantly raised mean value of SOD activity to 0.40 ± 0.03 U/mg protein compared to Vehicle.

**Bridelia ferruginea** methanol extract (BIME) improved Catalase (CAT) activity during experimental colitis: The mean values of CAT activity in rat’s colon tissue homogenate post administration of acetic acid is presented on Figs. 6. Mean activity of catalase (CAT) in colon tissue homogenate of control group was 7.09 ± 0.13Umg⁻¹ protein. acid significantly decreased the mean CAT activity in vehicle treated rats to 3.85 ± 0.36 Umg⁻¹ protein when matched with values obtained in the control animals. BIME significantly raised mean activity of CAT to 5.58 ± 0.34, 5.60 ± 0.49, and 6.06 ± 0.40 Umg⁻¹ protein at dosages of 100, 200, and 400 mg kg⁻¹ respectively, matched to vehicle. Sulfasalazine also, significantly increased mean CAT activity to 5.99 ± 0.42 U/mg protein compared to Vehicle.

**Figure 4:** Quantification of glutathione (GSH) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

**Figure 5:** Quantification of superoxide dismutase (SOD) activity during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

**Figure 6:** Quantification of catalase (CAT) activity during experimental colitis. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

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1.13 ± 0.86 pg/mg protein. BfME only slightly decreased mean TNF-α level to 2.84 ± 0.32 pg/mg protein at 100 mg kg⁻¹ dose, but at 200 and 400 mg kg⁻¹ dosages, TNF-α levels significantly reduced to 1.82 ± 0.13 and 1.83 ± 0.08 pg/mg protein respectively, compared to Vehicle. Sulfasalazine also significantly reduced mean level of TNF-α to 1.69 ± 0.20 pg/mg protein when matched with Vehicle.

**Figure 7:**
Quantification of tumor necrosis factor alpha (TNF-α) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

**Bridelia ferruginea** methanol extract (BfME) reduced I L-6 level during experimental colitis

The mean value of IL-6 levels in rat’s colon tissue homogenate post intrarectal administration of acetic acid are presented on Fig. 8. The mean value of IL-6 in the colon tissue homogenate of control group was 4.29 ± 0.31 pg/mg protein. Acid significantly increased mean IL-6 level to 8.72 ± 0.48 pg/mg protein in the Vehicle treated rats when compared with values in the control animals. BfME significantly decreased mean IL-6 levels to 6.80 ± 0.24, 6.77 ± 0.36, 6.42 ± 0.33 pg/mg protein at 100, 200 and 400 mg/kg dosages respectively, matched to vehicle. Sulfasalazine decreased mean IL-6 level to 6.10 ± 0.63 pg/mg protein compared to vehicle.

**Figure 8:**
Quantification of interleukin 6 (IL-6) levels during experimental colitis in rats. Values are given as Mean ± SEM (n = 4); # = significantly different from Control (p < 0.05); * = significantly different from Vehicle (p < 0.05), as determined by one-way ANOVA.

**Molecular docking**

For IL-6, the reference anti-inflammatory standard drugs prednisolone (binding energy of -5.6 kcal/mol) and celecoxib (binding energy of -5.7 kcal/mol) individually showed considerable binding energy, however, γ-sitosterol, α-amyrin, and stigmasterol, performed better than these standards with respective binding energy of -6.0 kcal/mol, -6.7 kcal/mol, and -5.8 kcal/mol respectively (Table 1). For iNOS, prednisolone displayed a binding energy of -6.5 kcal/mol, celecoxib displayed a binding energy of -7.8 kcal/mol, while α-amyrin showed a considerable binding energy of -8.1 kcal/mol (Table 1). For IL1-β, γ-sitosterol and α-amyrin with respective binding energy of -7.5 kcal/mol and -7.2 kcal/mol performed better than the two standards prednisolone and celecoxib which displayed -3.8 kcal/mol and -6.8 kcal/mol binding energy respectively (Table 1). With respect to TNF-α, γ-sitosterol, α-amyrin and stigmastaster also performed better than the standards with respective binding energy of -8.7 kcal/mol, -10.2 kcal/mol, and -8.3 kcal/mol respectively relative to -8.0 kcal/mol and -8.1 kcal/mol for prednisolone and celecoxib respectively (Table 1). In the case of COX-2, α-amyrin also displayed a higher binding energy of -7.7 kcal/mol relative to -7.4 kcal/mol and -6.9 kcal/mol for prednisolone and celecoxib respectively. These results showed that α-amyrin displayed higher binding affinity for all the five target proteins compared to prednisolone and celecoxib.

The interaction pattern of celecoxib with IL-6 include hydrogen bond with residue Lys-120, hydrogen bond with Glu-99, Pro-141, Asn-144,Tyr-346, py-alkyl bond with Leu-92, Pro-139, Ala-145 and Leu-148, while α-amyrin interacted with Leu-147 via py-alkyl bond (Plate 3).
Control shows a preserved mucosal epithelium in contrast to the Vehicle that is showing poorly preserved mucosa epithelium; ulcer and necrosis (white arrow), lamina propria shows suppurating inflammation and necrosis, the sub mucosal layer and muscularis layer show infiltration of inflammatory cells (blue arrow). Administration of BfME at the dosages of 200 and 400 mg kg\(^{-1}\) resulted in an improved epithelium as evidenced by a mild to moderately preserved mucosal epithelial layer, lamina propria is however, infiltrated by inflammatory cells. Sulfasalazine treatment also, attenuated inflammation as revealed by the moderately preserved mucosal epithelial layer (white arrow), lamina propria is however, infiltrated by inflammatory cells (slender arrow), the sub mucosal layer shows mild infiltration of inflammatory cells (blue arrow) and the serosal layer is also, inflamed and well vascularized (H & E Stain; *100).

### Table 1:

Docking results of major compounds identified in *Bridelia ferruginea* methanol extract with selected anti-inflammatory protein targets

<table>
<thead>
<tr>
<th>S/N</th>
<th>Compound</th>
<th>Binding affinity (kcal/mol)</th>
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<tr>
<td></td>
<td>IL-6</td>
<td>iNOS IL-1β</td>
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<tr>
<td>1</td>
<td>Prednisolone</td>
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<tr>
<td>2</td>
<td>Celecoxib</td>
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<td>3</td>
<td>Octadecenoic acid</td>
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<td>4</td>
<td>n-Hexadecenoic acid</td>
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<td>5</td>
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### Plate 3:

Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) with IL-6

### Plate 4:

Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) iNOS

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The interaction pattern of celecoxib with IL-6 includes hydrogen bond with residue Gln-265, Gly-288 and Trp-289, halogen bond with Tyr-264, py-sigma bond with Leu-287 while α-amyrin interacted with Trp-495 via pi-sigma (Plate 3). Also, Plate 5 shows the interaction pattern of celecoxib with IL-β include hydrogen bond with residue Leu-26, Leu-80, Leu-134, halogen bond with Tyr-24, Leu-80 and Val-132, pi-sigma bond with Leu-80, Pro-131 while α-amyrin interacted with Met-20 via pi-alkyl.

The interaction pattern of celecoxib with TNF-α was dominated by hydrogen bond, pi-alkyl bonds and halogen bond while only van der Waals forces could be observed for α-amyrin (Plate 6).

Plate 5:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) IL1-β (alkyl)

Plate 6:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) TNF-α

The interaction pattern of celecoxib with COX-2 include hydrogen bond with residue Lys-239, Thr-255, Gln-256, Arg-297 and Thr-547, halogen bond with Lys-543, pi-sigma bond with Leu-232 while α-amyrin interacted with Ile-301 via pi-alkyl (Plate 7).

Plate 7:
Amino acid interactions of reference inhibitor (celecoxib) and lead phytochemical (α-amyrin) COX-2

DISCUSSION

Induction of experimental ulcerative colitis by low application of acid via the rectum in rodents is recognizable simulation for studying IBD (George et al., 2020). Although this model and human IBD vary in etiology, they both have mutual pathophysiological descriptions and medication response in the course of treatment. Changes such as weight loss ulceration of the mucosa, blood loss and inflammation are examples of features that accompany them (Misbahuddin et al., 2021). Also, there is influx of inflammatory cells, rupture of colon mucosal barrier, release of inflammatory mediators and the production of reactive oxygen species (ROS) in both acid-induced colitis and IBD which results in decreased antioxidant system activity and consequently oxidative stress (Rehman et al., 2022; Sahoo et al., 2023).

In the present study, colitis was induced by intrarectal administration of 1 ml of acetic acid (4% v/v). Administration of vehicle (distilled water), BfME at 100, 200 and 400 mg kg⁻¹, and sulfasalazine (500 mg kg⁻¹) was done appropriately according to rat grouping and these commenced on the 3rd day post colitis induction till the 9th day when rats were euthanized.

In this study, significant inhibition of MDA levels in colon tissue homogenate of rats administered with BfME is indicative of its antioxidant and anti-inflammatory potentials. MDA, a by-product of lipid peroxidation occurring in tissues increases in inflammatory conditions such as in ulcerative colitis and thus, represent an important marker in the diagnosis of colitis (Rehman et al., 2022). Since lipid peroxidation increases during oxidative stress, administration of plant products such as BfME may be beneficial in synergy with endogenous antioxidant enzyme activities in reducing the production of oxidants.

Induction of colitis elevated the concentration of NO in this study; this was evidenced in the vehicle group. Administration of BfME however, significantly subdued NO generation which may have averted peroxynitrite reduction from inflammatory cells with consequent...
reduction in inflammation. Same effect of BfME on NO concentration in colon tissue homogenate of rats was observed with sulfasalazine administration. NO is considered a pro-inflammatory mediator when it is overproduced by means of inducible NO synthase. In such circumstances it can further increase inflammation to form a peroxynitrite (ONOO−), a powerful oxidant, through its reaction with superoxide anion (O2−) (Cross & Wilson, 2003). The acetic acid mediated increase in NO concentration was apparently suppressed by BfME in this study and suggests the extract’s ability to prevent neutrophil infiltration and inflammation in the colon as observed in the photomicrographs.

Myeloperoxidase (MPO) is a pro-inflammatory enzyme found in azurophilic granules of neutrophilic granulocytes which during stimulation produces highly reactive oxidants and cross-link proteins (Hoskin et al., 2019). BfME significantly attenuated the MPO activity, indicating the extract possesses anti-inflammatory properties that can target MPO during colitis, an action that may make it a potentially suitable therapeutic agent. Reduced glutathione (GSH) is a vital antioxidant mediator useful in the removal of free radicals that can cause mucosal damage (Vázquez-Meza et al., 2023). It plays crucial role in preserving the integrity of the epithelium against inflammation (Yaje et al., 2020). During inflammation there is oxidative stress and this causes a reduction in GSH concentration (Kumar et al., 2022). In this study, the induction of colitis with acid resulted in reduction of colonic tissue GSH concentration in rats administered with vehicle. However, BfME administration significantly attenuated the reduction in colonic tissue GSH concentration. The improvement of GSH concentration in rats administered with BfME may be indicative of its potentials for mopping up reactive oxygen species which in turn could attenuate acetic acid-induced oxidative damage in colon tissues.

In normal conditions, SOD and CAT are crucial in defense against oxidative stress in various disease conditions. In UC, activities of these duo in the tissues of the colon turn out to be intense and consequently they become depleted due to damage caused by free radicals (Vona et al., 2021). SOD protects colon tissues against colitis and consequently disallowed lipid peroxidation through the prevention of transformation of superoxide anion (Alyaa et al., 2021). SOD also disallowed leukocyte sticking together and penetration in the colon and as for CAT, it is concentrated in peroxisomes and they catalyze the change of H2O2 a cytotoxic agent to H2O and O2 (Baldo & Serrano, 2017). Administration of BfME prevented exhaustion of SOD and CAT activities thus, leading to overall protection of colonic tissues post acetic acid-induced experimental colitis. The opposite was however, observed in the rats that were administered with the vehicle.

Tumor necrosis factor alpha and IL-6 are inflammatory cytokines that play very important role in the pathophysiology of inflammatory conditions as they regulate mucosal immunity by altering the functions of the epithelium, activation and enhancement of cellular infiltrates like neutrophils and macrophages which then result in mucosal damage of the colon (Jang et al., 2021). The elevated values of IL-6 and TNF-α in rats administered with vehicle in contrast to their low values in rats administered with BfME further supports its anti-inflammatory potentials. A similar observation of low IL-6 and TNF-α values in rats administered with BfME was also noticed in the sulfasalazine group.

Histopathological assessment revealed that administration of BfME preserved the structural architecture and function of the intestinal colonic mucosa epithelium by disallowing inflammatory cell penetration, necrosis, hyperplasia ulceration triggered off by acetic acid, an indication of the ability of BfME to prevent progression of colonic inflammation.

Studies on molecular docking were carried out at the later end of the experiments to gain more insights about the promising activity profile of the compounds earlier identified in BfME (Onmolaso et al., 2021). Results from the docking study revealed outstanding binding affinity of α-amin for all evaluated anti-inflammatory protein targets, exhibiting superior binding affinity for IL-6, iNOS, IL1-β, TNF-α and COX-2 relative to prednisolone and celecoxib. Interestingly, earlier studies have established the anti-inflammatory activity of this pentacyclic triterpene. For example, α-amin and β-amin acetate isolated from the stem bark of Alstonia boonei displayed significant anti-inflammatory activity in egg albumen-induced paw edema and xylene-induced ear edema models in experimental animals (Akindele et al., 2020). Vitor and colleagues have also demonstrated the anti-inflammatory effect of α-amin in trinitrobenzene sulphonic acid-induced colitis in mice, relative to dexamethasone (Vitor et al., 2009), while Medeiros et al., highlighted its anti-inflammatory effect on phorbol ester 12-O-tetradecanoylphorbol-13-acetate induced skin inflammation of mouse skin (Medeiros et al., 2007). Stigmasterol displayed notable binding affinity with IL-6 and TNF-α while ɣ-Sitosterol showed notable binding affinity with IL-6, IL1-β and TNF-α. These compounds could be the major active components responsible for the observed anti-inflammatory activity of BfME in this study.

In conclusion, Bridelia ferruginea stem bark methanolic extract demonstrated antioxidative and anti-inflammatory activities in acetic acid-induced rats by restoring serum levels of NO, MDA, GSH, TNF-α, IL-6, and activities of MPO, SOD and CAT. Furthermore, α-amin from the methanolic extract showed various levels of binding affinities and molecular interactions with anti-inflammatory protein targets, including IL-6, iNOS, IL1-β, TNF-α and COX-2. These results may justify the folkloric use of this plant in the treatment of inflammatory conditions in traditional medicine.

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Anti-inflammatory effects of Bridelia ferruginea on acid-induced Ulcerative Colitis


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