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Evaluation of the Anti-Inflammatory and Antioxidant Properties of Flavonoid-Rich Seed Extract of *Buchholzia coriacea* Engler (Capparaceae)

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ABSTRACT

The anti-inflammatory and antioxidant potentials of the Buchholzia coriacea seed flavonoid-rich extract were evaluated. The *in vitro* anti-inflammatory studies for the extract were done using protease inhibition activity, membrane stabilization, and albumin denaturation inhibition assays, and the antioxidant activity was performed via determination of hydrogen peroxide (H_2O_2), nitric oxide (NO) and 2-2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assays as well as its total antioxidant capacity. The ethanol flavonoid-rich extract of *B. coriacea* seed (10 – 50 µg/ml) effectively inhibited the denaturation of albumin. The inhibition by the extract was concentration-dependent, with 10 µg/ml having an inhibition of 37.30 % and 50 µg/ml with the highest inhibition of 73.41%. The inhibitory ability of the extract on protease increased significantly ($p<0.05$) with increasing concentration. The extract at 50 µg/ml showed the highest inhibition of 50.08% compared to the standard drug at the same concentration, with inhibition of 39.54%. The flavonoid-rich extract of the seeds of *B. coriacea*, like indomethacin, significantly ($p<0.05$) protected the human erythrocyte membrane against lyses induced by hypotonic solution when compared to the control. The plant extract at different concentrations significantly ($p<0.05$) inhibited the oxidative stress caused by H_2O_2, DPPH, and NO radicals compared to the control. The extract demonstrated a good antioxidant capacity and thus could be employed as an antioxidant. The results obtained for the extract's *in vitro* anti-inflammatory, and antioxidant activities could be why it is used in folkloric medicine.

Keywords: Albumin Denaturation, Anti-Inflammatory, Antioxidant, Hydrogen Peroxide.

Introduction

Inflammation is a complex process frequently associated with pain and fever, involving increased vascular permeability, protein denaturation, and membrane alteration[1]. Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compounds such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat.[2] The clinical symptoms such as fever, aches, and pains associated with several diseases are directly or indirectly due to inflammatory disorders.[2] Most biological proteins lose their activities or functions when denatured. Denaturation of protein is a well-documented cause of inflammation.[3,4] Also, neutrophils are known to be a rich source of serine protease. It was previously reported that leukocyte protease plays an important role in tissue development and damage during inflammatory reactions, and a significant level of protection was provided by protease inhibitors.[5]

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Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, are among the most commonly recommended and prescribed medications for treating anti-inflammation worldwide.[6] Antioxidants are chemical compounds that can scavenge free radicals produced by normal physiological processes within the body. These free radicals start a chain reaction that leads to various other free radicals leading to oxidative stress, which in turn leads to the formation of reactive oxygen species and reactive nitrogen species, causing lipid peroxidation (LPO) and cellular harm. Such free radicals can also disintegrate various biomolecules, such as nucleic acids, proteins, and lipids.[7] Compounds demonstrating scavenging activity towards these ROS may have therapeutic potential for inflammatory diseases.

Buchholzia coriacea E. (Capparidaceae) is a forest tree with large, glossy, leathery leaves and conspicuous cream-white flowers in racemes at the end of the branches. The plant is easily recognized by the compound pinnate leaves and the long narrow angular fruits containing large, usually aligned seeds.[8] The plant is documented to possess diverse medicinal potential.[8] It has been used for years to treat a variety of illnesses. Okoli and colleagues reported the anti-plasmodial properties of the plant,[9] the ground seeds were, therefore, routinely mixed with palm oil and taken orally as a treatment for malaria.[10] The Cameroonians use the seed as a remedy to relieve chest pain.[11] It was also reported to have anti-inflammatory activity,[12] analgesic effects,[13] anthelminthic potentials,[14] anti-malarial,[15] antihelmintic activity,[16] antibacterial activity,[17] antimicrobial effects,[18] hypoglycemic effect,[19] anti-fertility potentials,[20] and anti-ulcer potentials.[21] Although the beneficial effects of *B. coriacea* seed extract have been exploited, no work has reported the anti-inflammatory potentials of the flavonoid-

rich extract of the seed; this study was therefore undertaken to evaluate the *in vitro* anti-inflammatory and antioxidant potentials of seed extract of *B. coriacea*.

Materials and Methods

Plant materials
Fresh Seeds of *B. coriacea* were collected in and around July 2021 from Ugwu-Awgbu in Orumba North LGA, Anambra State, Nigeria. The seed was identified and authenticated (Voucher number = INTERCEDD/16108) by Mr. Alfred Ozioko, a taxonomist with the Bioresources Development and Conservation Program (BDCP) Research Centre, Nsukka, Enugu State. The seeds were air-dried and pulverized.

Chemicals and reagents
All chemicals used in this study were of analytical grade and products of Sigma Aldrich, USA; British Drug House (BDH) England, Burgoyne, India; Harkin and Williams, England, Qualikems India, Fluka Germany, May and Baker England. Reagents used for the assays were commercial kits and products of Randox, USA and Teco (T.C.), USA.

Extraction procedure
The Fresh Seed of *B. coriacea* was collected and washed to remove dirt. The plant material was cut into bits and shade-dried with regular turning to avoid decaying. The dried seed was pulverized into the powdered form using a mechanical grinder. A known weight of the ground seed (1 kg) was macerated in 3.5 L absolute ethanol using a maceration flask. The mixture was left for 72 hours with occasional stirring, after which it was filtered into a flat-bottom flask using a muslin cloth. Further filtration was achieved with Whatman No 1 filter paper to remove fine residues. The filtrate was concentrated using a rotary evaporator at 45 ℃ to obtain the crude ethanol extract. The concentrated extract was stored in a labeled sterile screw-capped bottle in a refrigerator.

Preparation of flavonoid-rich Extract of B. coriacea seeds
Extraction of the flavonoid-rich Extract of the seeds of *B. coriacea* was carried out according to the method described by Chu *et al*.[22] Exactly 3 g of the crude extract was dissolved in 20mL of 10 % H_2SO_4 in a small flask and was hydrolyzed by heating in a water bath for 30 minutes at 100^{o}C. The mixture was placed on ice for 15 minutes to allow for the precipitation of the flavonoid aglycones. The cooled solution was filtered, and the filtrate (flavonoid aglycone mixture) was dissolved in 50 mL of warm 95% ethanol (50^{o}C). The resulting solution was again filtered into a 100 mL volumetric flask made up to the mark with 95 % ethanol. The filtrate collected was concentrated to dryness using a rotary evaporator. The process was repeated several times to obtain a good quantity of the flavonoid-rich extract of the seeds of *B. coriacea* for the experiments

Biochemical assays
Effect of the flavonoid-rich Extract on albumin denaturation
Using the method of Mizushin and Kobyashi[23] with minor modifications, the reaction mixture consisted of the test extracts (10 – 50 µg/ml) and a 1 % aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of HCl at 37^{o}C. The sample extract was incubated at 37^{o}C for 20 minutes and then at 51^{o}C for 20 minutes. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. 20 µg/ml diclofenac sodium was used as the control. The experiment was performed in triplicate. Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \text{x } 100$$

Effect of flavonoid-rich Extract of B. coriacea seeds on protease inhibition
The test was performed according to Oyedepo and Femurewa[24] and Sakat *et al.*[25] with minor modifications. The reaction mixture (2 ml) contained 0.06 mg trypsin, 20 Mm Tris-HCl buffer (pH 7.4), and a 1 ml test sample of each of the different concentrations (0.1 - 0.5 µg/ml). The mixture was incubated for an additional 20 minutes. 2 ml of 70 % perchloric acid was added to arrest the reaction. The cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against the buffer as blank. 50 µg/ml diclofenac sodium was used as the control. The experiment was performed in triplicate. The percentage inhibition of protease inhibitory activity was calculated as follows:

$$\text{Percentage inhibition (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) x100$$

Effect of the flavonoid-rich Extract of B. coriacea seeds on hypotonicity-induced haemolysis of human red blood cells
This was determined using modifications of the method of Oyedepo *et al*.[26]

Preparation of human red blood cells suspension
A blood sample (3 ml) obtained from a healthy volunteer was placed into an EDTA bottle, centrifuged at 3,000 rpm for 10 min, and washed three times with an equal volume of normal saline. The blood volume was measured and reconstituted with normal saline as a 40 % (v/v) suspension.

Procedure
Samples of the Extract and indomethacin used were dissolved in distilled water, serving as the hypotonic solution. An aliquot (1 ml) of varying concentrations of the extract (0.1, 0.2, 0.4, 0.6, 0.8 mg/ml) was put into each of a set of five test tubes. Another tube contained 1 ml of 0.6 mg/ml indomethacin. The contents of the respective tubes were made up to 4.9 ml with the vehicle (distilled water). Two control tubes were used for this test. A control tube contained 4.9 ml of the vehicle, while another contained 4.9 ml of normal saline (isotonic solution). HRBCs suspension (0.1 ml) was added to each tube, and after gentle mixing, the mixtures were incubated for 1 h at room temperature (370^{o}C). After incubation, the reaction mixture for each tube was centrifuged at 3,000 g for 10 min, and the absorbance of the supernatant was measured at 418 nm using a spectrophotometer. The tests were carried out in triplicates. Reaction media containing 1ml varying concentrations of extract or indomethacin made up to 5.0 ml in normal saline, without HRBCs suspension, were used as the respective blank for each test. The blank for the control tubes contained normal saline also without HRBCs suspension. The percentage inhibition of hemolysis was calculated using the relation:

$$\text{\% Inhibition of Haemolysis} = 100\left(1 - \frac{OD2 - OD1}{OD3 - OD1}\right)$$

Where;
OD_1 = absorbance of control I (isotonic solution)
OD_2 = absorbance of the test sample
OD_3 = absorbance of control II (hypotonic solution)

In vitro antioxidant assay
Hydrogen peroxide scavenging activity
This plant extract activity was evaluated by Ruch *et al.*[27] Eighty (80) µL of the aqueous plant extract was added to 150 µL of 4 Mm hydrogen peroxide solution prepared in phosphate buffer (0.1 M, pH 7.4). This was incubated for 10 minutes, and the absorbance was read at 230 nm.

$$\text{Percentage inhibition (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) x\ 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Nitric oxide (NO) scavenging activity
Nitric oxide scavenging activity was by the method of Garrat.[28] The sample was determined by adding 400 µL of 100 mM sodium nitroprusside, 100 µL of PBS (pH 7.4), and 100 µL of different concentrations of the plant extract. This reaction mixture was kept for incubation at 250 ^{o}C for 150 minutes. To 0.5 ml of the above solution,

0.5 ml of Griess reagent was added (0.1 ml of sulfanilic acid and 200 µL) anphthylethlenediamine dichloride (0.1 %w/v). This was kept incubating at room temperature for 30 minutes, and finally, the absorbance was read at 540 nm. All the reactions were performed in triplicates, and the formula calculated their percentage inhibition:

$$\text{Percentage inhibition (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) x100$$

Determination of DPPH radical scavenging activity
DPPH (2-2- diphenyl-1-picrylhydrazyl) was used to determine the free radical scavenging activity of the extract by the method of Bloiss.[29] DPPH radical was prepared by dissolving 0.0024 g DPPH in 100 ml methanol (purple). To 1.0 ml of different concentrations from 0.1 - 0.6 µg/ml, 1.0 ml DPPH was added and left in the dark for 20 minutes, and then the absorbance was read at 517 nm. Ascorbic acid was used as a reference drug/control. The percentage inhibition of DPPH formation was calculated as follows:

$$\text{Percentage inhibition (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) x\ 100$$

Determination of total antioxidant capacity
The total antioxidant capacity (TAC) of the extract was determined by the phosphomolybdate method as previously reported by Sakat *et al.*[25] Briefly, 1 ml aliquot of various concentrations of the extract (0.1-0.6 µg/ml) was mixed with 1 ml of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate, 1:1:1). The test tubes were cooled to room temperature. The absorbance of the mixture was determined at 765 nm against a blank containing 1 ml of the reagent solution. Ascorbic acid was used as standard. The assay was carried out in triplicates. The total antioxidant capacity (TAC) was expressed as mg equivalent of ascorbic acid per gram (EAA/g). The total antioxidant capacity of the extract was estimated as follows:

$$\text{Total antioxidant capacity (\%)} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) x\ 100$$

Statistical analysis
The data obtained were analyzed using one-way variance analysis (ANOVA) in Statistical Product and Service Solution (SPSS) version 23.0. The values are presented as Mean ± S.D. and considered significant at $p < 0.05$.

Results and Discussion

The study was carried out to investigate the *in vitro* anti-inflammatory and antioxidant activity of the flavonoid-rich Extract of *B. coriacea* seeds. It was carried out using albumin denaturation, protease inhibition, membrane stabilization, hydrogen peroxides (H_2O_2), 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) radical scavenging activities, and total antioxidant capacity approaches as study models.

Effects *of flavonoid-rich Extract of B. coriacea seed on albumin denaturation*
Inflammation is the reaction process of living tissues to stimuli evoked by inflammatory agents such as physical injuries, heat, microbial infections, and toxic chemical irritations. The response of cells toward inflammation will lead to specific pathological manifestations characterized by redness, warmth, swelling, and pain with even impaired physiological functions. Inflammation has been implicated in the pathogenesis of many diseases, including arthritis, stroke, and cancer.[30] Protein denaturation has been well correlated with the occurrence of the inflammatory response and leads to various inflammatory diseases, including arthritis.[31] According to Opie, tissue injury during life might be preferable to denaturation of the protein constituents of cells or intercellular substances.[32] Hence, the ability of a substance to inhibit the denaturation of protein signifies the apparent potential for anti-inflammatory activity. The ethanol flavonoid-rich extract of *B. coriacea* seeds effectively inhibited albumin denaturation, as shown in Table 1. Different concentrations of the extract inhibited significantly ($p < 0.05$) albumin denaturation compared to the control. The standard drug (diclofenac sodium, a routinely used NSAID for arthritis) followed a similar trend. The inhibition by the extract was concentration-dependent, with 10 µg/ml having an inhibition of 37.3 % and 50 µg/ml with the highest inhibition of 73.41 %. This assay has therefore provided evidence for its good anti-inflammatory properties.

Effects of the flavonoid-rich Extract of B. coriacea seed on protease activity
Proteases have been implicated in arthritic reactions. Neutrophils are a rich source of serine protease and are localized at lysosomes. It was previously reported that leukocyte protease plays an essential role in the development of tissue damage during inflammatory reactions, and protease inhibitors provide a significant level of protection.[5] The Ethanol seed extract of *B. coriacea* significantly ($p < 0.05$) inhibited the protease activity at different concentrations than the control, as shown in Table 2. The standard drug (50 µg/mL diclofenac sodium) followed a similar trend. The inhibiting ability of the extract increased significantly with increased concentration. The extract at 50 µg/ml showed the highest inhibition of 50.08 % compared to the standard drug (diclofenac sodium) at the same concentration, with inhibition of 39.54 %. This bioactivity could be attributed to the extract's high flavonoid content.

Table 1: Effects of flavonoid-rich Extract of *B. coriacea* seed on albumin denaturation

Treatment	Concentration (µg/mL)	Absorbance (O.D) 660 nm	% Inhibition
Control	-	0.504 ± 0.003^{e}	-
Extract 1	10	0.316 ± 0.002^{d}	37.30
Extract 2	20	0.239 ± 0.053^{c}	52.58
Extract 3	30	0.174 ± 0.002^{b}	65.48
Extract 4	40	0.174 ± 0.002^{b}	70.83
Extract 5	50	0.134 ± 0.004^{a}	73.41
Diclofenac Sodium	20	0.232 ± 0.001^{c}	53.97

n = 3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Table 2: Inhibitory effects of flavonoid-rich Extract of *B. coriacea* seed on protease activity

Treatment	Concentration (µg/mL)	Absorbance (O.D) 210 nm	% Inhibition
Control	-	1.214 ± 0.003^{g}	-
Extract 1	10	0.896 ± 0.001^{f}	26.19
Extract 2	20	0.819 ± 0.001^{e}	32.54
Extract 3	30	0.806 ± 0.001^{d}	33.61
Extract 4	40	0.792 ± 0.001^{c}	34.76
Extract 5	50	0.606 ± 0.003^{a}	50.08
Diclofenac Sodium	50	0.734 ± 0.003^{b}	39.54

n = 3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Effect of the flavonoid-rich Extract of B. coriacea seeds on membrane stabilization

The flavonoid-rich extract of *B. coriacea* seed exhibited a high membrane stabilization effect against hypotonicity-induced haemolysis of the red cells, as shown by the level of inhibition of haemolysis. As shown in table 3, the flavonoid-rich extract of the seeds of *B. coriacea*, like indomethacin, significantly ($p < 0.05$) protected the human erythrocyte membrane against lyses induced by hypotonic solution when compared to the control; the inhibition exhibited by extract is in a concentration-dependent manner, increasing with the increasing amount of the extract in the medium, with 0.8 µg/ml of the extract having the highest inhibition of 82.10 %. Its activity was also comparable to indomethacin, a standard anti-inflammatory drug. Protection against hypotonicity-induced haemolysis is related to membrane stabilization, an anti-inflammatory index.[33] Hypotonicity-induced haemolysis of human red blood cells (HRBC) occurs due to water uptake by the cells and leads to the release of haemoglobin which absorbs maximally at 540 nm. Hence, the reduced optical density at 540 nm obtained for the various *B. coriacea* seed test samples reflected the stabilization of the red cell membrane caused by the extract. The extract may also inhibit processes that stimulate or enhance intracellular components' efflux. The erythrocyte membrane is analogous to the lysosomal membrane.[34,35] Its stabilization implies that *B. coriacea* seed extract may stabilize lysosomal membranes against the release of lytic enzymes. Lysosomal enzymes play an important role in developing acute and chronic inflammation. Most anti-inflammatory drugs exert their beneficial effects by either inhibiting the release of enzymes or stabilizing the lysosomal membranes.[35] Stabilization of lysosomal membranes is important in preventing the leakage of serum protein and fluids into the tissue during increased permeability caused by inflammatory mediators. The anti-haemolytic properties of the flavonoid-rich Extract of *B. coriacea* seeds may be due to some active constituents such as flavonoids, tannins, and saponins. It has been reported that flavonoids exert profound stabilizing effects on lysosomes *in vitro* and *in vivo* in experimental animals.[36,37,38]

Hydrogen Peroxide (H_2O_2) radical scavenging activity of the flavonoid-rich extract

From table 4 below, the plant extract at different concentrations (10, 20, 30, 40, 60 µg/ml) inhibited significantly ($p < 0.05$) oxidative stress caused by hydrogen peroxide (H_2O_2) radicals when compared to the control. The percentage inhibition exhibited by the extract is in a concentration dependent manner; as the concentration of the extract (10, 20, 30, 40, 60 µg/ml) increases, the percentage inhibition increases having 23.8, 44.0, 57.8, 73.1, 86.2% respectively, as the standard (ascorbic acid) having percentage inhibition 86.2%. Hydrogen peroxide (H_2O_2) is highly important because of its ability to penetrate biological membranes. The scavenging of H_2O_2 radicals by the extract may be attributed to the flavonoids, which can donate electrons to H_2O_2, thus neutralizing it to water.[39] The results showed that the flavonoid-rich Extract of *B. corriacea* seeds had potent H_2O_2 scavenging activity, possibly due to the antioxidant compounds therein. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O.

DPPH (2-2-Diphnyl-1-Picrylhydrazyl) radical scavenging activity of the flavonoid-rich extract

The plant extract at different concentrations (10, 20, 30, 40, 60 µg/ml) inhibited significantly ($p < 0.05$) oxidative stress caused by DPPH radicals when compared to the control (Table 5). The percentage inhibition exhibited by the extract is in a concentration dependent manner; as the concentration of the extract (10, 20, 30, 40, 60 µg/ml) increases, the percentage inhibition increases having 24.8, 45.9, 49.5, 56.9, 59.2 % respectively, the standard (ascorbic acid) having percentage inhibition 57.8 %. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of an antioxidant.[40] When DPPH gains the hydrogen atom from the antioxidant compounds, it leads to a color change; the color change is directly proportional to the inhibitory activity of the antioxidant compound. It shows the inhibitory activity is due to the maximum hydrogen-donating ability of *B. coriacea* flavonoid-rich seed extract.

The DPPH scavenging assay results reveal that the flavonoid-rich Extract of *B. coriacea* seeds was potently active. This results from the decrease in the mean absorbance values and the increase in the percentage inhibition of DPPH radical scavenging activities with increasing extract concentration.

Table 3: Effect of the flavonoid-rich Extract of *B. coriacea* seeds on hypotonicity Induced Haemolysis in Red Blood Cell

Treatment	Conc. (µg/mL)	(O.D) 540 nm	OD_2-OD_1	% Inhibition
Control	-	0.432 ± 0.001^{h}	-	-
Control	-	0.432 ± 0.001^{h}	-	-
Extract 1	0.1	0.243 ± 0.002^{g}	0.135	58.33
Extract 2	0.2	0.233 ± 0.002^{f}	0.125	61.42
Extract 3	0.4	0.215 ± 0.001^{d}	0.107	66.98
Extract 4	0.6	0.178 ± 0.002^{c}	0.070	78.40
Extract 5	0.8	0.166 ± 0.006^{b}	0.058	82.10
Indomethacin	0.6	0.225 ± 0.004^{e}	0.117	63.89

n=3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Table 4: Hydrogen peroxide scavenging activity of flavonoid-rich Extract of *B. coriacea* seeds

Treatment	Concentration (µg/mL)	Absorbance (O.D) 230 nm	% Inhibition
Control	-	1.804 ± 0.002^{g}	-
Extract 1	10	1.374 ± 0.005^{f}	23.8
Extract 2	20	1.010 ± 0.003^{e}	44.0
Extract 3	30	0.764 ± 0.005^{d}	57.6
Extract 4	40	0.486 ± 0.003^{c}	73.1
Extract 5	60	0.249 ± 0.002^{a}	86.2
Vit C. (Ascorbic Acid)	100	0.361 ± 0.002^{b}	80.0

n=3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Table 5: 2-2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity of flavonoid-rich Extract of *Buchholzia Coriacea* seeds

Treatment	Concentration (µg/mL)	Absorbance (O.D) 517 nm	Percentage Inhibition
Control	-	0.218 ± 0.003^{e}	-
Extract 1	10	0.164 ± 0.004^{d}	24.8
Extract 2	20	0.118 ± 0.002^{c}	45.9
Extract 3	30	0.110 ± 0.002^{b}	49.5
Extract 4	40	0.094 ± 0.002^{a}	56.9
Extract 5	60	0.089 ± 0.003^{a}	59.2
Vit C. (Ascorbic Acid)	100	0.092 ± 0.005^{a}	57.8

n=3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

The extract's ability to significantly ($p > 0.05$) inhibit DPPH scavenging activity could be due to the hydroxyl group existing in the flavonoid-rich compounds' chemical structure that can provide necessary components as a radical scavenger.

NO radical scavenging activity of the flavonoid-rich extract

The plant extract at different concentrations (10, 20, 30, 40, 60 µg/ml) inhibited significantly ($p < 0.05$) oxidative stress caused by Nitric oxide (NO) radicals when compared to the control, as shown in Table 6. The percentage inhibition exhibited by the extract is in a concentration-dependent manner; as the concentration of the extract (10, 20, 30, 40, and 60 µg/ml) increases, the percentage inhibition increases having 20.4, 29.9, 36.2, 48.8, and 49.4% respectively, the standard (ascorbic acid) having percentage inhibition 50.9%. Nitric oxide (NO) is a free radical. It changes the structure and functions of the cellular membranes. It is formed from sodium nitroprusside and reacts with free radicals to form nitrite. The antioxidant compound directly reacts with the free radicals, and other nitrogen compounds prevent nitric oxide formation. This may prevent cellular damage. In the present study, the inhibition of NO formation by flavonoid-rich seed extract of *B. Coriacea* was observed, and the inhibition was exhibited in a concentration-dependent manner.

Table 6: Nitric oxide (NO) radical scavenging activity of flavonoid-rich Extract of *B. coriacea* seeds

Treatment	Concentration (µg/mL)	O.D 540 nm	% Inhibition
Control	-	1.123 ± 0.004^{g}	-
Extract 1	10	0.894 ± 0.003^{f}	20.4
Extract 2	20	0.787 ± 0.002^{e}	29.9
Extract 3	30	0.717 ± 0.003^{d}	36.2
Extract 4	40	0.575 ± 0.003^{c}	48.8
Extract 5	60	0.568 ± 0.003^{b}	49.4
Vit.C (Gallic Acid)	100	0.551 ± 0.002^{a}	50.9

n=3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Table 7: Total antioxidant capacity of flavonoid-rich Extract of *B. corriacea* seeds.

Treatment	Concentration (µg/mL)	O.D 765 nm	% Inhibition
Control	-	1.162 ± 0.001^{g}	
Extract 1	10	0.785 ± 0.003^{f}	32.4
Extract 1	20	0.755 ± 0.005^{e}	35.0
Extract 1	30	0.737 ± 0.006^{d}	36.6
Extract 1	40	0.723 ± 0.002^{c}	37.8
Extract 1	60	0.695 ± 0.002^{a}	40.2
Vit. C (Gallic Acid)	100	0.702 ± 0.007^{b}	39.6

n=3, result expressed as Mean ± S.D, mean values with different lowercase letters as superscripts across the groups are considered significant at ($p < 0.05$).

Total antioxidant capacity (TAC) of flavonoid-rich Extract of B. coriacea seeds

The plant extract at different concentrations (10, 20, 30, 40, and 60 µg/ml) increased in a concentration-dependent manner compared to the control (Table 7). Also, the percentage inhibition exhibited by the extract is in a concentration-dependent manner; as the concentration of the extract (10, 20, 30, 40, 60 µg/ml) increases, the percentage inhibition increases having 32.4, 35.0, 36.6, 37.8, 40.2 % respectively, the standard (ascorbic acid) having percentage inhibition of 39 %. The results showed that the flavonoid-rich Extract of *B. coriacea* seeds had good antioxidant activities due to the flavonoids' presence.[41]

Conclusion

The flavonoid-rich extract was found to possess radical scavenging and anti-inflammatory activities as determined by the albumin denaturation, protease inhibition, membrane stabilization, hydrogen peroxides (H_2O_2), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO) radical scavenging activities, and total antioxidant capacity data. Further research on the isolation, purification, and characterization of the particular class of flavonoids responsible for the observed actions is necessary. It could pave the way for incorporation into existing anti-inflammatory herbal compositions, improving their efficacy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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