

Proceeding Paper

Therapeutic Evaluation of the Antioxidant Capacity of Flavonoid-Rich Seed Extract of *Buchholzia coriacea* Engler (Capparaceae) †

Osmund Chukwuma Enechi ¹, Okeke Emmanuel Sunday ^{1,2,3,*}, Nkwoemeka Ndidi Ethel ^{2,4} and Ezeorba Chidike Prince Timothy ¹

¹ Department of Biochemistry, University of Nigeria, Nsukka 410001, Enugu State, Nigeria; osmundcenechi@gmail.com (O.C.E.); timothy.ezeorba@unn.edu.ng (E.C.P.T.)

² Natural Science Unit, School of General Studies, University of Nigeria, Nsukka 410001, Enugu State, Nigeria; nkwoemeka@unn.edu.ng

³ School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China

⁴ Department of Microbiology, University of Nigeria, Nsukka 410001, Enugu State, Nigeria

* Correspondence: emmanuel.okeke@unn.edu.ng; Tel.: +2348035277554.

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Abstract: Inflammation is a complex process frequently associated with pain and fever, involving increased vascular permeability, increased protein denaturation, and alteration of the membrane. The clinical symptoms such as fever, aches, and pains associated with several diseases are directly or indirectly due to inflammatory disorders. Most biological proteins lose their biological activities or functions when denatured. An evergreen shrub which is distributed in African countries including Nigeria, among others, belonging to the family of Capparidaceae has been used in traditional medicine as a valuable alternative therapy in the treatment of Malaria, asthma and cough, diarrhoea, rheumatism, ulcers, worm infection, diabetes, hypertension, psychiatric disorders, and impotence. This work studies the anti-inflammatory and antioxidant performance of a flavonoid-rich extract of *Buchholzia coriacea* Engler (Capparaceae) seed. The in vitro anti-inflammatory studies for the extract were done using protease inhibition activity, membrane stabilization, and albumin denaturation inhibition assays, and antioxidant activities were performed by determination of hydrogen peroxide (H₂O₂), nitric oxide (NO), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assays as well as total antioxidant capacity. The ethanol flavonoid-rich extract of *B. coriacea* seed was effective in inhibiting the denaturation of albumin in a concentration-dependent manner. The inhibitory ability of the extract on protease increased significantly ($p < 0.05$) with increased concentration. 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 inhibited significantly ($p < 0.05$) oxidative stress caused by H₂O₂, DPPH, NO radicals when compared to the control. The flavonoid-rich extract was found to possess radical scavenging and anti-inflammatory activities as determined by albumin denaturation, protease inhibition, membrane stabilization, hydrogen peroxides (H₂O₂) 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO) radical scavenging activities, and total antioxidant capacity data. Further research on isolating, purifying, and characterizing the particular class of flavonoids responsible for the aforementioned activities may be undertaken, and they may be incorporated into existing anti-inflammatory herbal compositions to improve their efficacy.

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1. Introduction

Inflammation is a complex process frequently associated with pain and fever, involving occurrences such as increased vascular permeability, increased protein denaturation, and alteration of the membrane (Leelaprakash and Mohama, 2010). Furthermore, neutrophils are known to be a rich source of serine proteases and play an important role in the development of tissue damage during inflammatory reactions, and consequently, protease inhibitors provide a significant level of protection (Korkmaz et al., 2010). 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. The clinical symptoms such as fever, aches, and pains associated with several diseases are directly or indirectly due to inflammatory disorders (Okeke et al., 2019). Most biological proteins lose their biological activities or functions when denatured. Denaturation of protein is a well-documented cause of inflammation (Leelaprakash and Mohama 2010; Ingle and Patel, 2011). Also, neutrophils are known to be a rich source of serine protease and it was previously reported that leukocytes protease play an important role in the development of tissue damage during inflammatory reactions and a significant level of protection was provided by protease inhibitors (Das and Chatterjee, 2010). Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, are among the most commonly recommended and prescribed drugs for treating anti-inflammation in the world. 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 the formation of 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. Compounds that demonstrate scavenging activity towards these ROS may have therapeutic potential for inflammatory diseases.

Buchholzia coriacea E. (Capparidaceae), known as musk tree, is a forest tree with large, glossy, leathery leaves and conspicuous cream-white flowers in racemes of 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. The plant is documented to possess diverse medicinal potentials. It has been used for years to treat a variety of illnesses. Okoli and colleagues reported the anti-plasmodial properties of the plant (Okoli et al., 2010), the ground seeds were therefore routinely mixed with palm oil and taken orally as a treatment for malaria (Adjanohoun et al., 1996). The Cameroonians use the seed as a remedy to relieve chest pain (Thomas et al., 1989). It was also reported to have anti-inflammatory activity (Enechi et al., 2019), analgesic effects (Ezeja et al., 2011), anthelmintic potentials (Nweze and Asuzu, 2006), anti-malarial (Enechi et al., 2016), antihelmintic activity (Ajaiyeoba et al., 2001), antibacterial activity (Mbata, Duru and Onwumelu, 2009), antimicrobial effects (Ezekiel and Onyeoziri, 2009), hypoglycemic effect (Adisa et al., 2011), anti-fertility potentials (Obembe et al., 2012) and anti-ulcer potential (Enechi and Nwodo, 2014). Although the beneficial effects of *B. coriacea* seed extract have been exploited, no work has been reported on 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*.

2. Materials and Methods

2.1. Plant Materials

Fresh Seeds of *B. coriacea* were collected from Ugwu-Awgbu in Orumba North LGA, Anambra State, Nigeria. The seed was identified and authenticated by Mr. Alfred Ozioko, a taxonomist with the Bioresources Development and Conservation Program (BDGP) Research Centre, Nsukka, Enugu State. The seeds were air-dried and pulverized.

2.2. 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 (TC), USA.

2.3. Extraction Procedure

The Fresh Seed of *B. coriacea* was collected and washed to remove dirt. The plant material was cut into pieces and shade-dried with regular turning to avoid decaying. The dried seed was pulverized into a powdered form using a mechanical grinder. A known weight of the pulverized seed (1 kg) was macerated in 3.5 L absolute ethanol using a maceration flask. The mixture was left for 72 h 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 °C to obtain the crude ethanol extract. The concentrated extract was stored in a labeled sterile screw-capped bottle at 2–8 °C.

2.4. Preparation of Flavonoid-Rich Extract of *B. coriacea* Seeds

In 20 mL of 10% H₂SO₄ in a small flask, exactly 3 g of the crude extract was dissolved and was hydrolyzed by heating on a water bath for 30 min at 100 °C. The mixture was placed on ice for 15 min, to allow the precipitation of the flavonoids aglycones. The cooled solution was filtered and the filtrate (flavonoid aglycone mixture) was dissolved in 50 mL of warm 95% ethanol (50 °C). The resulting solution was again filtered into a 100 mL volumetric flask which was made up to the mark with 95% ethanol. Using a rotary evaporator, the filtrate collected was concentrated to dryness (Chu et al., 2002).

2.5. Biochemical Assays

2.5.1. Hydrogen Peroxide Scavenging Activity

This activity of the plant extract was evaluated by the method of (Ruch et al., 1989)

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

Where A₀ is the absorbance of the control and A₁ is the absorbance of the sample.

2.5.2. Nitric Oxide (NO) Scavenging Activity

Nitric oxide scavenging activity was by the method of Garrat (1964), The percentage inhibition was calculated by the formula below;

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

2.5.3. 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 (1958). The percentage inhibition of DPPH formation was calculated as follows:

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

2.5.4. Determination of Total Antioxidant Capacity

The total antioxidant capacity (TAC) of the extract was determined by the phosphomolybdate method as previously reported (Sakat et al., 2010). 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:

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

2.6. Statistical Analysis

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

3. Results

3.1. Hydrogen Peroxide (H₂O₂) Radical Scavenging Activity of the Flavonoid-Rich Extract

From Table 1 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 (H₂O₂) 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%.

Table 1. Hydrogen Peroxide Scavenging Activity of Flavonoid-Rich Extract of *B. coriacea* Seeds.

Treatment	Concentration (µg/mL)	O.D230nm	O.D230nm
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 ($p < 0.05$).

3.2. DPPH (2-2-Diphnyl-1-Picrylhydrazyl) Radical Scavenging Activity of the Flavonoid-Rich Extract

From Table 2 below, 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. The percentage inhibition exhibited by the extract is in 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%.

Table 2. 2-2-Diphnyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity of Flavonoid-Rich Extract of *B. coriacea* Seeds.

Treatment	Concentration (µg/mL)	O.D 517nm	%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	0.094 ± 0.002 ^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).

3.3. 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 3 below. The percentage inhibition exhibited by the extract is in concentration dependent-manner; as the concentration of the extract (10, 20, 30, 40, 60 µg/mL) increases, the percentage inhibition increases having 20.4, 29.9, 36.2, 48.8, 49.4% respectively, the standard (ascorbic acid) having percentage inhibition 50.9%.

Table 3. Nitric Oxide (NO) Radical Scavenging Activity of Flavonoid-Rich Extract of *B. coriacea* Seeds.

Treatment	Concentration (µg/mL)	D540nm	%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	0.575 ± 0.003 ^c	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).

3.4. Total Antioxidant Capacity (TAC) of Flavonoid-Rich Extract of *B. coriacea* Seeds

The plant extract at different concentrations (10, 20, 30, 40, 60 µg/mL) increased in a concentration-dependent manner when compared to the control. Also, the percentage inhibition exhibited by the extract is in 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%. This is shown in the Table 4 below.

Table 4. Total Antioxidant Capacity of Flavonoid-Rich Extract of *B. coriacea* Seeds.

Treatment	Concentration (µg/mL)	D765nm	%Inhibition
Control		1.162 ± 0.001 ^g	
Extract 1	10	0.785 ± 0.003 ^f	32.4
Extract 2	20	0.755 ± 0.005 ^e	35.0
Extract 3	30	0.737 ± 0.006 ^d	36.6
Extract 4	40	0.723 ± 0.002 ^c	37.8
Extract 5	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).

4. Discussion

The present 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, H₂O₂, DPPH, NO radical scavenging activities, and total antioxidant capacity approaches as study models.

H₂O₂ is highly important because of its ability to penetrate biological membranes (Di Marzo et al., 2018). Scavenging of Hydrogen peroxide radicals by the extract may be attributed to the flavonoids, which can donate electrons to H₂O₂, thus neutralizing it to water (Ebrahimzadeh et al., 2010). The results showed that the flavonoid-rich extract of *B. coriacea* seeds had potent H₂O₂ scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H₂O₂ to H₂O. Thus, in the present study, the flavonoid-rich extract of *B. coriacea* seeds was capable of scavenging H₂O₂ in a concentration-dependent manner

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of an antioxidant (Chung et al., 2006). When DPPH gains the hydrogen atom from the antioxidant compounds, it leads to a colour change; the colour 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 result of the DPPH scavenging activity assay reveals that the flavonoid-rich extract of *B. coriacea* seeds was potently active. This is because; as there was a decrease in the mean absorbance value and an increase in the percentage inhibition of DPPH radical scavenging activity with increased extract concentration. The ability of the extract 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, a gas compound produced by an enzyme NO synthase, is possessed with different physiological and biological effects on the body (Papi et al., 2029). As a free radical, it plays a major role in the aggregation of platelets, blood flow, cytotoxicity, synaptic transmission, and neurotransmitters. It also changes the structure and functions of the cellular membranes (Kurutas, 2016). The antioxidant compounds which directly react with the free radicals and other nitrogen compounds prevent nitric oxide formation. This may prevent cellular damages (Lobo et al., 2010). In the present study, the inhibition of NO formation by flavonoid-rich seeds extract of *B. coriacea* was observed and the inhibition was exhibited in a concentration-dependent manner.

The results obtained from total antioxidant capacity showed that the flavonoid-rich extract of *B. coriacea* seeds had good antioxidant activities which could be due to the presence of flavonoids (Rice-Evans et al., 1999). Therefore, flavonoids are considered as having good antioxidant properties.

5. Conclusions

The flavonoid-rich extract was found to possess radical scavenging, hydrogen peroxides (H₂O₂), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO) radical scavenging activities, and total antioxidant capacity data. Further research on isolating, purifying, and characterizing the particular class of flavonoids responsible for the aforementioned activities may be undertaken and they may be incorporated into existing antioxidant herbal compositions to improve their efficacy.

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Conflict of Interest: None declared.

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