Tanzania Journal of Science
Volume 51(4), 2025
journal homepage: https://commons.udsm.ac.tz/tjs/

Antioxidant and anti-inflammatory effects of *Blighia unijugata* Baker aril protein-rich extract

Oludele O Odekanyin*, Ifeoluwa M Adepoju, Tolulope A Anjorin, and Oluwatobi S Adekoya
Biochemistry and Molecular Biology Department, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

Keywords

Antioxidant;
aril proteins;
Blighia unijugata;
anti-hemolytic

Abstract

The study investigated the *in vitro* oxidative-stress-mitigating effect and anti-inflammatory potential of the soluble protein of the arils of *Blighia unijugata* in order to establish possible use in inflammatory therapy. Air-dried *Blighia unijugata* aril was pulverized into powder, defatted using petroleum ether, and homogenized in Phosphate Buffer Saline. The homogenate was centrifuged, and the supernatant was subjected to ammonium sulphate fractionation. The precipitate was thoroughly dialyzed, dialysate was freeze-dried, and termed partially purified *Blighia unijugata* aril protein extract (ppBUAPE). The antioxidant potential of the protein-rich extract was investigated using DPPH, H_2O_2 scavenging activity, and FRAP. The ability of ppBUAPE to inhibit protease (trypsin) and lipoxygenase activities, as well as its antihemolytic property, was investigated to elucidate its mechanism of inflammatory treatment. The protein extract possessed antioxidant potential. The extract scavenged both radical (DPPH) and non-radical (H_2O_2) oxidants. Its DPPH scavenging activity, as shown by half-maximal inhibitory concentration (IC_{50}) of 0.84 ± 0.18 mg/ml, was significantly lower than standard (ascorbic acid) IC_{50} of 0.22 ± 0.06 mg/ml, while H_2O_2 scavenging ability (IC_{50} of 96.06 ± 5.39 µg/ml) was comparable to standard (IC_{50} of 96.46 ± 8.21 µg/ml) potential. It displayed low iron-reducing power. Protein extracts inhibited trypsin and lipoxygenase activities with IC_{50} of 27.53 µg/ml and 1.56 mg/ml, respectively. ppBUAPE displayed a dose-dependent antihemolytic activity comparable to that of diclofenac (standard). The research provides in vitro evidence for the oxidative-stress mitigating and anti-inflammatory effects of the ppBUAPE.

Introduction

Oxidative stress is a result of an imbalance created by excessive generation of reactive oxygen species (ROS), which the endogenously produced antioxidants cannot neutralise or eradicate. Under this condition, biomolecules like proteins, nucleic acids, and lipids are attacked by these free radicals, leading to cellular disorder, cell death, and inflammation (Gulcin 2020). Inflammation is a natural immune response to tissue degeneration caused by noxious stimuli, physical damage/injury to cells or tissues, and microbial infection (Gusev and Zhuravleva 2022). It is the mechanism employed by the body to eliminate invading microorganisms, to neutralize the harmful chemical agents, and pave the way for the rebuilding of damaged tissue. Inflammatory processes initiated by oxidative damage or stress have been recognized as major contributors to the pathogenesis of several chronic diseases and some metabolic disorders, such as arthritis, aging, diabetes, Alzheimer's disease, cancer, atherosclerosis, and Parkinson's disease (Chaudhary et al. 2023, Leyane et al. 2022). The scavengers of ROS, found mostly in natural products, play major roles in preventing oxidative stress-related diseases.

The antioxidants, which are inherent in the phytochemicals present in plant materials, are increasingly being sought after to alleviate the injurious activities of ROS (Muscolo et al. 2024). Aside, secondary metabolites like saponins, alkaloids, and flavonoids that have been the centre of focus for various pharmacological activities, proteins and their derivatives have also been reported to be good sources of antioxidant, anti-inflammatory, and other health-beneficial potential (Dada et al. 2023, Derbel et al. 2023, Salami et al. 2023, Adewole et al. 2024). The natural products, especially proteins or their hydrolysates, can be a good source for the development of new drugs with good antioxidant potential coupled with anti-inflammatory activity. Availability of plants, perceived efficacy, pocket-friendliness, and no injurious side effects are the merits of herbal products over synthetic antioxidants (Okaiyeto and Oguntibeju 2021).

Blighia unijugata Baker, commonly referred to as Triangle top in English, Akoko-Isin or Isin-Oko in Yorubaland, Southwest Nigeria, Oja-wala in Igboland,

*Corresponding author: dekanyin@oauife.edu.ng
Received 27th February 2025, Revised 9th June 2025, Accepted 27th November 2025, Published 24 December 2025
https://doi.org/10.65085/2507-7961.1107

ISSN 0856-1761, e-ISSN 2507-7961

and Ukpe-nchwi in Edoland, both of Southeastern Nigeria, is an underutilised tree. It is mostly found in West African countries such as Nigeria, Congo, and Côte d'Ivoire, referred to as the African ackee, and it is widely spread across countries in sub-Saharan Africa (Burkill 2000). *Blighia unijugata* is a small tree with a good-looking appearance. The fruit is red or pinkish red with three black seeds carrying a yellowish-coloured aril. The plant is usually 30-35m in height and purposely planted as a shaded tree (Burkill 2000). It belongs to the Sapindaceae Family, and it is abundantly used in folk medicine. It is used in the treatment and management of high blood pressure, arthritis, cough, stiffness of joints, eye discomfort, and renal pain (Burkill 2000). The leaf of *B. unijugata* has also been employed as an ecbolic and abortifacient. Literature has shown that the plant possesses some pharmacological activities like insecticidal, antioxidant, anti-inflammatory, anti-bacterial, and antidiabetic (Ajiboye et al. 2017, Osuala et al. 2020, Oloyede et al. 2023). The leaf, root, stem, and bark possess sedative and analgesic activities.

These therapeutic properties are due to the presence of phytochemicals in the leaf, root, and stem bark of the plant. Polyphenols, flavonoids, saponins, steroids, cardiac glycosides, tannins, and alkaloids have been reported in the plant (Ajiboye et al. 2017). Despite the focus and interest on the research of *B. unijugata,* much information is not available on the biological activity of proteins from various anatomical parts of the plant. The major uses of its seeds and fruit pods are to produce oil and potash for soap making. Oderinde et al. (2008) reported the proximate analysis of aril and kernel, and stated that crude protein content is 19.90% and 14.00% respectively. Likewise, protein content of the leaves of *B. unijugata* was reported to be 24.13% (Offor et al. 2014). However, there is little or no scientific investigation that has been published to establish the therapeutic and pharmacological potential of protein extracts or isolates from any part of this plant.

Considering the adequate amount of crude protein present in *B. unijugata* aril (Oderinde et al. 2008), which is highly nutritious, the current study aims to further scientifically substantiate the traditional uses of this plant for oxidative stress-related diseases. Specifically, the study focuses on obtaining protein-rich extract from *B. unijugata* arils and investigating its antioxidant and anti-inflammatory potentials. This will provide information in support of the consumption of arils, establish the health benefits of the arils, and lay the foundation for the isolation of therapeutic agents from protein sources, which might be useful in anti-inflammatory and other related disease treatment.

Material and Methods

Collection of plant materials

Blighia unijugata fruits were harvested around the Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun state, Nigeria. The plant was identified and authenticated by the herbarium curator in the Ife Herbarium, Botany Department, OAU, Ile-Ife. The sample of the plant was deposited in the herbarium with Voucher number (IFE18289).

Preparation of crude protein extract

Blighia unijugata pods were opened, and arils were separated from the seeds. The arils were blended into a paste and suspended repeatedly in several changes of petroleum ether to remove oil. The oil-free paste was air-dried at room temperature. Fifty grams of the dried paste was weighed and suspended in 0.02M Phosphate buffer containing 0.1 M NaCl (PBS, pH 7.2) in 1 to 10 (w/v) ratio. The mixture was put in a conical flask and agitated on a magnetic stirrer at 4 ºC for 24 hours. The homogenate was later centrifuged at 10,000 rpm for 20 minutes. The supernatant was termed *B. unijugata* arils crude protein extract.

Ammonium sulphate precipitation

The *Blighia unijugata* aril crude protein extract was subjected to salt precipitation (70% ammonium sulphate saturation) and left for about 18 hours at room temperature for the formation of the precipitate. The mixture was centrifuged to collect the precipitate, which was redissolved in a minimal volume of PBS (pH 7.2) and later dialyzed against PBS extensively. The resulting solution after dialysis was centrifuged to remove debris. The obtained supernatant was lyophilized and termed partially purified *Blighia unijugata* aril protein extract (ppBUAPE), which was used for various biological activity assays (Odekanyin et al. 2017).

Protein concentration determination by Lowry's method

Protein content of the crude extract and partially purified extract was determined using the Lowry method described by Rizvi et al. 2022. Bovine serum albumin (BSA) was employed as a standard to develop a standard protein curve.

Assessment of antioxidant potential of partially purified protein.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging potential of ppBUAPE was evaluated using the amended method of Blois (1958) described by Sadiq et al. (2023). DPPH working solution (0.1 mM DPPH in 95% methanol) was added to 1 mg/ml of various volumes (0.05 – 1.0 ml) of partially purified BUAPE. The test tubes were placed in the darkroom for 30 min at 25 ºC. Reading was taken at 517 nm wavelength in the Biobase UV-visible spectrophotometer BK-D5 series. The blank has distilled water in place of the ppBUAPE, and ascorbic acid was used as the standard antioxidant agent. The equation below was used to calculate the DPPH radical scavenging ability of ppBUAPE in percentage.

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

Hydrogen peroxide (H_2O_2) radical scavenging assay

The hydrogen peroxide (H_2O_2) radical scavenging capability of ppBUAPE was carried out based on the amended protocol of Ruch et al. (1989). Working solution

of H_2O_2 (40mM) was made in sodium phosphate buffer (pH 7.4). Reaction mixture of 2.5 ml contains various volumes (0.0 – 0.6 ml) of 5mg/ml ppBUAPE and 1.5 ml of the H_2O_2 working solution in test tubes. The absorbance was read at 230 nm wavelength in a UV/Visible spectrophotometer (Biobase UV-visible spectrophotometer BK-D5 series) after incubating the reaction mixture for 10 min at ambient temperature. The blank solution contains distilled water as a negative control. Aspirin was employed as a positive control.

$$H_2O_2 \text{ Scavenging } (\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

Ferric reducing antioxidant power (FRAP) assay

The procedure of Benzie and Strain (1996) of assaying for ferric reducing antioxidant power was adopted with slight modifications for the evaluation of ppBUAPE's ability to reduce ferric ion to ferrous ion. FRAP reagent, prepared just before use, is made up of 20 mM $FeCl_3.6H_2O$, 10 mM 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine (TPTZ, dissolved in 40 mM HCl), and Acetate buffer (300 mM, pH 3.6) in the ratio of 1:1:10, respectively. Known volumes of 1.0 mg/ml ppBUAPE were pipetted and mixed with FRAP reagent. The mixture was vigorously shaken, incubated for 10 mins, and the optical density was taken in the darkroom at ambient temperature using a 593 nm wavelength. The absorbance was compared with the negative control containing distilled water. Reducing antioxidant power was expressed as equivalent concentration (EC), which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard.

Assessment of anti-inflammatory potential of the extract

Antiprotease assay

The ability of partially purified *Blighia unijugata* protein isolate to inhibit protease activity was investigated using trypsin as the enzyme and N-benzoyl DL-arginine p-nitroanilide (BAPNA) as the chromogenic chemical substrate. The protocol was published by Vasudev and Sohal (2016). Briefly, 210 µl of 50 mM Tris-HCl buffer (pH 8.0), 280 µl of 2 mM BAPNA solution (dissolved in 50 mM Tris-HCl buffer), and varying concentrations (0.0 - 0.3 mg/ml) of ppBUAPE are mixed in a reaction mixture of 700 µl. The addition of 10 µl 1 mg/ml trypsin solution (dissolved in 1 mM HCl) starts the reaction, and a change in absorbance is recorded every 30 sec for 4 mins. Enzyme, inhibitor, and substrate blanks were prepared appropriately. Residual activity of the enzyme was calculated using the equation below;

$$\text{Residual activity} = \frac{OD/\min \times Total\ volume\ of\ assay\ mixture}{9.96\ \text{mM}-1\text{cm}-1 \times \text{Enzyme volume}}$$

Where 9.96 $mM^{-1}cm^{-1}$ is the molar extinction coefficient.

The percentage inhibition of trypsin was obtained as shown below;

$$\text{Percentage inhibition of trypsin} = 100 - \frac{\text{Activity of trypsin in the presence of inhibitor}}{\text{Activity of trypsin in the absence of inhibitor}} \text{ x } 100$$

Lipoxygenase inhibitory assay

Lipoxygenase inhibitory activity of ppBUAPE with linoleic acid as substrate was measured with a UV/visible spectrophotometer as described by Konate et al. (2011) with some modifications. Extracts at varying concentrations were screened for lipoxygenase inhibitory activity. The assay mixture consisted of 150 µl phosphate buffer (60 mM, pH 7.5), 50 µl of extract solution at varying concentrations, and 50 µl enzyme solution (167 U/ml in phosphate buffer). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in the same buffer). Enzymatic kinetics were recorded at 234 nm for 5 min. Negative control was prepared and contained 1% methanol solution without the extract solution. Quercetin at varying concentrations was used as the positive control. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase activity, calculated as (%) inhibition using the following formula:

$$\% \text{ inhibition} = \frac{\Delta\text{Abs control} - \Delta\text{Abs sample}}{\Delta\text{Abscontrol}} \text{ x } 100$$

Anti-hemolytic assay

The human blood group O-positive used in this assay was first prepared as follows. The blood was drawn from a healthy donor and washed five times with PBS using centrifugation. Five percent (5%) of the washed erythrocytes was then prepared in PBS. Ebrahimzadeh et al. (2010) procedure was adopted with little modification to determine the anti-hemolytic activity. 500 µl of different concentrations of ppBUAPE/standard (Ascorbic acid) were added to the erythrocyte suspension in PBS (5%, 1.0 ml). After 30 min incubation, 1.0 ml of H_2O_2 (1.77 M) was added to the mixture. Incubation for 3 hours at 37°C followed by gentle shaking of the mixture. After incubation, the reaction mixture was centrifuged for 10 min at 2500 rpm. The supernatant was collected, and then the absorbance was read at 540 nm to estimate the amount of hemoglobin released. The erythrocytes were also treated as above, but without the protein extract to obtain complete hemolysis taken as 100%.

Data analysis

The data obtained were expressed as the mean ± standard deviation. Each experiment was conducted in triplicate. Statistical analysis was done using GraphPad Instat Software version 8.0.

Results

The potential of ppBUAPE to scavenge both radical (DPPH) and non-radical (H_2O_2) oxidants was tested. The assay revealed that the ability of the ppBUAPE to neutralize oxidants is dose-dependent (Figure 1). However, the ability of ppBUAPE (IC_{50} = 0.84 ± 0.18 mg/ml) to scavenge DPPH, a radical oxidant, markedly

differs from that of the standard scavenger, ascorbic acid (IC_{50} = 0.22 ± 0.06 mg/ml), which is four times greater.

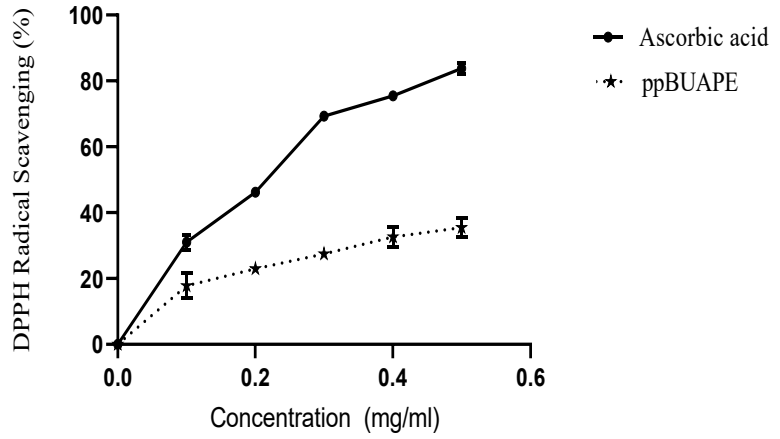

Figure 1: DPPH Radical Scavenging Activity of Ascorbic Acid and ppBUAPE

The non-radical scavenging potential of ppBUAPE is also dose dependent (Figure 2). The capability of ppBUAPE and aspirin to scavenge H_2O_2 is similar, as indicated by their IC50 values. Aspirin resulted in an IC_{50} of 96.46 ± 8.21 µg/ml, and ppBUAPE gave an IC_{50} of 96.06 ± 5.39 µg/ml.

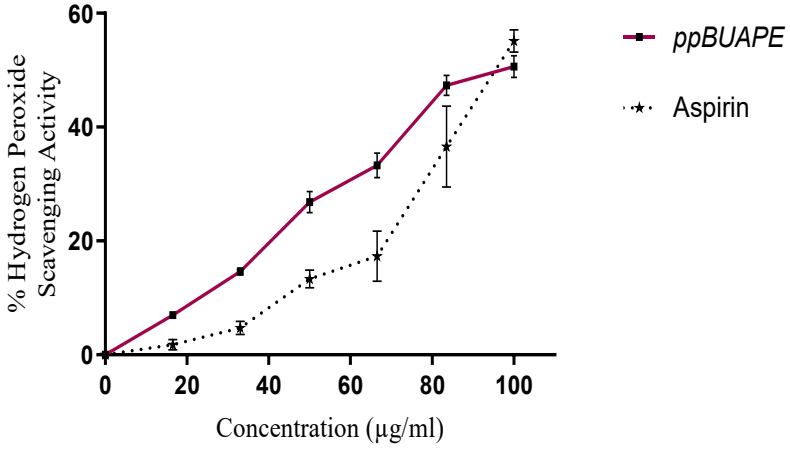

Figure 2: Hydrogen peroxide scavenging activity of *Blighia unijugata* aril protein extract and standard (aspirin)

The ppBUAPE metal-reducing antioxidant potential was measured in milligrams of ascorbic acid equivalent per gram of the protein extract. ppBUAPE exhibited a low reducing antioxidant power of 9.76 ± 0.80 mg ascorbic acid per gram protein extracts.

Inhibition of protease (trypsin) and lipoxygenase activities, as well as prevention of hemolysis, were used to investigate the anti-inflammatory activity of the protein-rich extract of *Blighia unijugata* arils. The protease inhibitory activity of ppBUAPE was examined using a kinetic assay method. The results showed that trypsin hydrolysis of BAPNA was greatly inhibited by ppBUAPE, with an IC_{50} of 27.53 µg/ml. The result is depicted in Figure 3, and it shows that the antiprotease potential depends on the concentration of the protein extract.

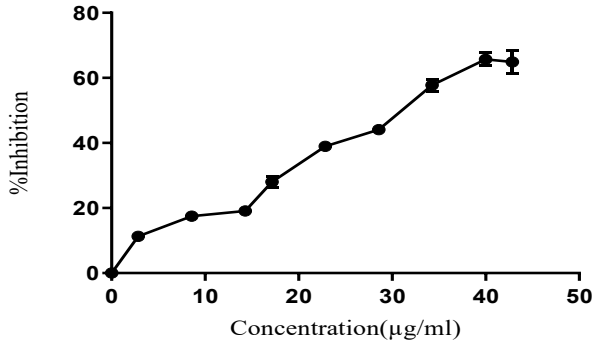

Figure 3: Trypsin inhibitory activity of *Blighia unijugata* aril protein extract

Lipoxygenase activity inhibition, as shown in Figure 4, revealed that ppBUAPE inhibitory action on lipoxygenase is directly proportional to concentration, and its lipoxygenase inhibitory action is lower compared to the effect of quercetin on lipoxygenase activity. At 0.025 mg/ml of quercetin, 55.76±1.53% of lipoxygenase activity was inhibited, while at 2.5 mg/ml of ppBUAPE, a 100-fold increase in concentration, 61.98±4.12% of lipoxygenase activity was inhibited.

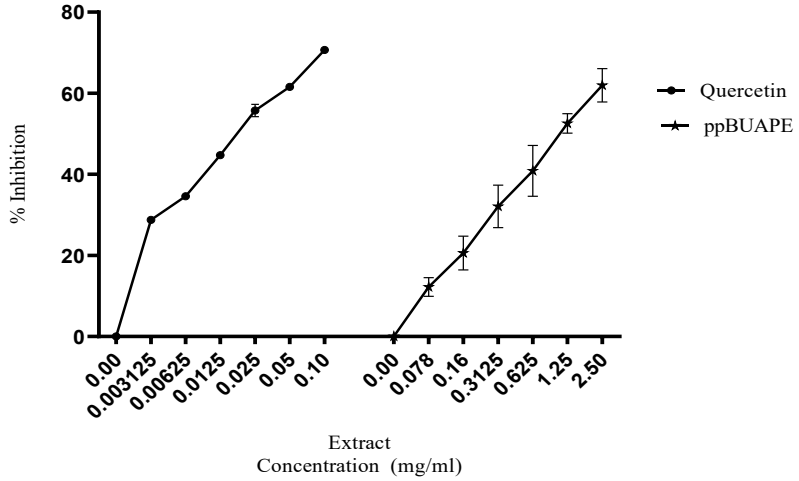

Figure 4: Lipoxygenase activity inhibition by Quercetin standard) and *Blighia unijugata* aril protein extract

The ppBUAPE displayed a dose-dependent anti-hemolytic ability (Figure 5). The activity was comparable to the membrane-stabilizing effect of the standard drug (Diclofenac) used. The IC_{50} obtained for the extract (IC_{50} = 0.816 ± 0.18 mg/ml) is higher than that of the standard drug (IC_{50} = 0.675 ± 0.07 mg/ml). This indicated that the extract is less effective in protecting the membrane from oxidative attack.

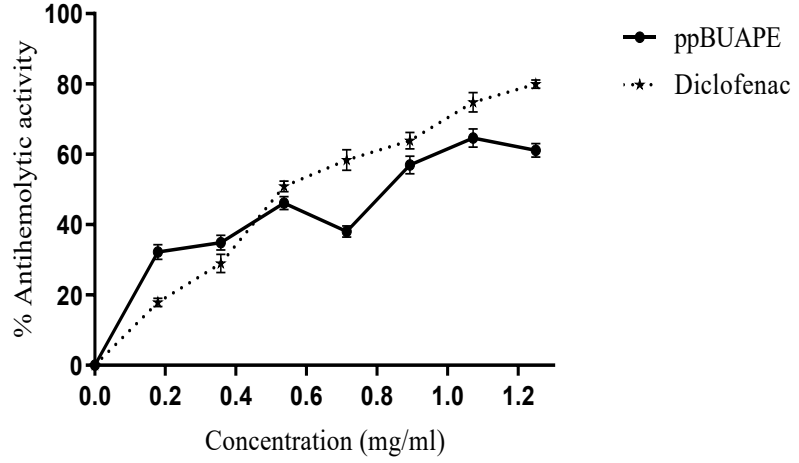

Figure 5: Anti-hemolytic activity of *Blighia unijugata* aril protein extract

Discussion

Blighia unijugata is traditionally used in Africa for folk medicinal treatment and management of ailments such as high blood pressure, arthritis, cough, joint stiffness, eye

discomfort, and renal pain. Evidence from Burkill (2000), Ojewole et al. (2005), and Osuala et al. (2020) supports the traditional medicinal use of *B. unijugata*: its aqueous and methanolic stem-bark extracts exhibit anti-inflammatory activity. Fiadjoe and Koffuor (2024) and Frédéric et al. (2021) studied the toxic effects of ethanolic extract of *B. unijugata* stem bark and butanoic extract of *B. unijugata* leaves on the haematological parameters, to establish the plant consumption safety. Antioxidant (Ajiboye et al. 2017), anticancer (Matata et al. 2018), anti-insecticidal (Oloyede et al. 2023), and hypoglycemic (Mohammed et al. 2023) activities are other scientific evidence that support the *B. unijugata's* various extracts efficacy. We embarked on this study to further substantiate, scientifically, the traditional uses of this plant for the treatment of oxidative stress-related diseases. The aril, the only consumable part of the fruit, was used in this research because little scientific research is available, though *Blighia sapida* arils have been extensively worked on (Dossou et al. 2014, Ibrahim et al. 2023, Maigida and Bukar 2024, Nabede et al. 2025).

The nutritious, ripe arils were extracted with phosphate-buffered saline to obtain crude soluble protein at neutral pH, thereby maintaining the structural integrity of the proteins. The crude soluble protein was fractionated with ammonium sulphate to concentrate the proteins due to their differential solubility in salt solution. The fraction contained a measurable amount of protein, substantiating the early report of Oderinde et al. (2008) on the presence of 19.90% crude protein in *B. unijugata* arils.

A wide range of assays, each based on distinct mechanisms, is available for evaluating antioxidant activity. According to Elias et al. (2008), the antioxidant potential of proteins arises from complex interactions involving their capacity to inactivate reactive oxygen species, scavenge free radicals, chelate pro-oxidative transition metals, reduce hydroperoxides, and enzymatically eliminate specific oxidants. As such, the antioxidant capability of an extract, particularly one rich in proteins or peptides, cannot be reliably determined using a single assay. To address this, the present study employed three in vitro models, namely DPPH radical scavenging, hydrogen peroxide scavenging, and ferric reducing antioxidant power (FRAP) assays, to comprehensively assess the antioxidant activity of ppBUAPE.

The DPPH radical scavenging assay is a substrate-free system, and its simplicity, combined with the rapidity of analysis, makes it one of the most widely employed methods for evaluating antioxidant activity. The result shows that ppBUAPE antioxidant prowess is directly proportional to the concentration of the protein (Figure 1). However, its efficacy was markedly lower compared to the standard ascorbic acid. This was also revealed by the half-maximal inhibitory concentration (IC50) of 0.84 ± 0.18 mg/ml for ppBUAPE, which is about four times the IC_{50} of ascorbic acid (0.22 ± 0.06 mg/ml). Yet, the ability of ppBUAPE to scavenge DPPH radical is similar to the value reported for crude protein and peptides obtained from *Rhodomonas* sp (Derbel et al. 2023). The antioxidant displayed by ppBUAPE may be due to the availability of amino acids involved in free radical deactivation, and also the ability to release a hydrogen atom easily to DPPH radical. These attributes conferred on ppBUAPE can neutralize radicals, thereby protecting humans against diseases.

Hydrogen peroxide is a non-free radical that is capable of disrupting the membrane layer because of its oxidizing power. A highly reactive hydroxyl radical ($OH^{\cdot}$), which can initiate lipid peroxidation, oxidize protein and other organic matter, and cause DNA damage in the body, can be generated from it. The toxicity of hydrogen peroxide is due to the hydroxyl radical ($OH^{\cdot}$). It is therefore essential for the biological system to eliminate or reduce the amount of H_2O_2 in the system. Scavenging of H_2O_2 by antioxidants from the cell reduces its availability for conversion to hydroxyl radical and its toxic effect on the cell. In this study, the non-radical scavenging potential of ppBUAPE is a function of concentration. As the amount of ppBUAPE rises, the scavenging activity also goes up (Figure 2). The capability of ppBUAPE and aspirin to scavenge H_2O_2 is similar, as indicated by their IC_{50} values. Aspirin resulted in an IC_{50} of 96.46 ± 8.21 µg/ml, and ppBUAPE gave an IC_{50} of 96.06 ± 5.39 µg/ml. Compared to the H_2O_2 scavenging activity reported by Moualek et al. (2016) for *Arbutus unedo* aqueous extract (IC_{50} of 114.77 ± 16.81 µg/ml) ppBUAPE showed to have a better scavenger.

The ability of ppBUAPE to reduce ferric-TPTZ to ferrous form was also used to measure its antioxidant activity. The ppBUAPE metal-reducing antioxidant potential was measured in milligrams of ascorbic acid equivalent per gram of the protein extract. ppBUAPE exhibited a low reducing antioxidant power of 9.76 ± 0.80 mg ascorbic acid per gram protein extracts. The reaction was conducted at low pH, and an increase in absorbance due to the production of an intense blue color of ferrous-TPTZ complex was monitored at 593 nm. Rubio et al. (2016) stated that this assay method has an edge over other methods in that the assay is highly reproducible over a wide range of concentrations, low cost, and the sample needs no pre-treatment. It is also very fast and simple. Reducing power of a plant extract is a reflection of electron-donating capacity and antioxidant activity concepts. The antioxidant molecules present in the ppBUAPE play a reductant role and cause the reduction of the Fe^{3+}/TPTZ complex to the ferrous form.

Anti-inflammatory activity of the protein-rich extract was assessed through its potential to inhibit the activity of proteases and lipoxygenase and its ability to protect cell membrane integrity. Serine proteases are major components of neutrophil lysosomal constituents. They are abundantly present in neutrophil lysosomal granules. Their release, along with other constituents during the inflammation process, causes serious tissue damage that has been shown/noticed in some diseases (Famutimi et al. 2024). This group of enzymes has been implicated in the reaction that leads to arthritis. Protease inhibitors provide protective succour from further harm to tissue by the proteases. Hence, antiprotease activity can be used as a measure of anti-inflammatory activity. ppBUAPE showed significant ability to inhibit the activity of trypsin, a member of the serine protease family. The antiprotease activity of the protein extract was dose-dependent, with an IC_{50} value of 27.53 ± 2.11 µg/ml. Consequently, the results suggest the presence of a bioactive principle in

ppBUAPE that may contribute to its anti-inflammatory potential. Derbel et al. (2023) reported anti-inflammatory activity for bioactive proteins and peptides from *Rhodomonas* sp using the protein denaturation mechanism. They reported a 67% inhibitory effect at 500 µg/ml for crude protein but this was increased with enzymatic hydrolysis of the protein. At a lower concentration of 50 µg/ml, ppBUAPE exhibited 65.7% inhibitory effects, revealing that protein-rich extract is ten-fold better than peptides from *Rhodomonas* sp (Derbel et al. 2023).

Lipoxygenases are therapeutic targets for some human diseases because their metabolites, especially leukotrienes, which are biologically active and are involved in the stimulation of inflammatory and allergic reactions (Hu and Ma 2021). The correlation between inflammation and diseases such as cancer, neurodegenerative and cardiovascular, allergic, and strokes has been studied (Hu and Ma 2021). Therefore, compounds or phytochemicals capable of inhibiting lipoxygenase activity may play a crucial role in preventing inflammation and disease, and thus represent a potential therapeutic strategy for the management of inflammatory conditions. ppBUAPE exhibited concentration-dependent inhibition of lipoxygenase activity but its effect was significantly varied from the quercetin standard. When compared with the anti-lipoxygenase activity of various plant extracts reported by Lončarić et al. (2021), the observed effect falls within the range documented in their review.

Stabilization of membrane architecture and inhibition of cell lysis have often been used as measures of the anti-inflammatory capability of biological extracts (Yesmin et al. 2020). The reactive oxygen species generated at the point of injury, as well as an increase in volume in the area, disturb membrane fluidity, cause cell lysis, and eventually release lysosomal content, which includes proteases and other pro-inflammatory agents. The uncontrolled activation of these processes and the sustained production of free radicals during inflammation can lead to chronic inflammation, which has been implicated in the onset and progression of several neurodegenerative and metabolic diseases (Yesmin et al. 2020). Any biological or chemical agents that can maintain the integrity of the cell membrane as well as reduce or totally inhibit cell destruction are considered to be anti-inflammatory mediators. The red blood cell membrane, which is considered a resemblance to the lysosomal membrane, was subjected to chemical oxidative attack using H_2O_2. It was found that the protein extracts stabilized the RBC membrane subjected to chemical oxidative stress and inhibited the cell hemolysis. The stabilization of the membrane and inhibition of hemolysis were directly proportional to the concentration of the ppBUAPE. The highest antihemolytic activity was observed at 1 mg/ml with 64.6%. The standard, diclofenac, produced a higher hemolysis inhibition at the same concentration and exhibited a better membrane stabilization overall, with an IC_{50} of 0.675 mg/ml against ppBUAPE, with an IC_{50} value of 0.816 mg/ml.

Conclusion

The present in-vitro study indicates that a partially purified aril protein extract from *Blighia unijugata* (ppBUAPE) mitigates oxidative and inflammatory processes primarily via radical and hydrogen-peroxide scavenging, along with inhibition of trypsin and lipoxygenase. Consistent with its low FRAP value, the antioxidant effect appears to rely more on scavenging than on redox (metal-ion) reduction. These findings provide biochemical support consistent with the plant's traditional medicinal use. Future work should isolate the active protein(s)/peptide(s), quantify potencies with confidence intervals and curve-fit parameters, and evaluate in-vivo efficacy and safety before therapeutic conclusions are drawn.

Conflict of interest: All authors declare no conflicts of interest.

References

Adewole TS, Bieni MC, Ogundepo GE, Odekanyin OO and Kuku A 2024 Investigation of functional, antioxidant, anti-inflammatory, and antidiabetic properties of legume seed protein hydrolysates. *Food Hydrocoll. Health* 5(2): 100175.

Ajiboye CO, Moronkola DO and Adesomoju AA 2017 DPPH free radical scavenging activities of leaf, stem bark, root, flower and fruit of *Blighia unijugata* Baker (Sapindaceae) extracts. *J. Chem. Biol. Phys. Sci.* 7(4): 1190-1197.

Benzie IF and Strain JJ 1996 The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239(1): 70-76.

Blois M 1958 Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* 181: 1199–1200.

Burkill HM 2000 The Useful Plants of West Tropical Africa: Families S-Z. Royal Botanic Gardens; United Kingdom. 2nd Edition.

Chaudhary P, Janmeda P, Docea A, Yeskaliyeva B, Abdull Razis AF, Modu B, Calina D and Sharifi-Rad J 2023 Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Front. Chem.* 11: 1158198.

Dada SO, Ehie GC, Osukoya OA, Anadozie SO, Adewale OB and Kuku A 2023 In vitro antioxidant and anti-inflammatory properties of *Artocarpus altilis* (Parkinson) Fosberg (seedless breadfruit) fruit pulp protein hydrolysates. *Sci. Rep.* 13(1): 1493.

Derbel H, Elleuch J, Mahfoudh W, Michaud P, Fendri I and Abdelkafi S 2023 In Vitro Antioxidant and Anti-Inflammatory Activities of Bioactive Proteins and Peptides from *Rhodomonas* sp. *Appl. Sci.* 13(5): 3202.

Dossou VM, Agbenorhevi JK, Combey S and Afi-Koryoe S 2014 Ackee (*Blighia sapida*) fruit arils: Nutritional phytochemicals and antioxidant properties. *Int. J. Food. Sci. Nutr*. 3(6): 534-537.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM and Eslami B 2010 Antihemolytic and antioxidant activities of *Allium paradoxum*. *Cent. Eur. J. Biol.* 5: 338-345.

Elias RJ, Kellerby SS and Decker EA 2008 Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr.* 48(5): 430-441.

Famutimi OG, Adebiyi VG, Akinmolu BG, Dada OV and Adewale IO 2024 Trypsin, chymotrypsin and

elastase in health and disease. *Futur. J. Pharm. Sci.* 10: 126.

Fiadjoe HK and Koffuor GA 2024 Effect of ethanolic stem-bark extract of *Blighia unijugata* on body and organ weight, biochemistry, and hematology in Sprague-Dawley rats. *J. Drug Deliv. Ther.* 14(1): 65-72.

Frédéric NK, Narcisse GB, Bernard GN and Paul YA 2021 Effects of repeated administration of butanolic fraction of *Blighia unijugata* Bak. (Sapindaceae) leaves on some hematological parameters in Wistar rats. *J. Med. Plants Stud.* 9(4): 129-135.

Gulcin İ 2020 Antioxidants and antioxidant methods: An updated overview. *Arch. Toxicol.* 94(3): 651-715.

Gusev E and Zhuravleva Y 2022 Inflammation: A new look at an old problem. *Int. J. Mol. Sci.* 23(9): 4596.

Hu C and Ma S 2017 Recent development of lipoxygenase inhibitors as anti-inflammatory agents. *Med. Chem. Commun.* 9(2): 212-225.

Ibrahim AA, Idris M, Yakubu A and Ibrahim UF 2023 Proximate and Mineral Compositions of Flours of Ackee Seed and Aril from Bida Area of Niger State, Nigeria. *Lapai J. Sci. Technol.* 9(1): 94-105.

Konate K, Souza A, Therese KY, Dibala IC, Barro N, Millogo-Rasolodimby J and Nacoulma OG 2011 Phytochemical composition, antioxidant and anti-inflammatory potential of bioactive fractions from extracts of three medicinal plants traditionally used to treat liver diseases in Burkina Faso. *Int. J. Phytomed.* 3: 406-415.

Leyane TS, Jere SW and Houreld NN 2022 Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. *Inter. J. Mol. Sci.* 23(13): 7273.

Lončarić M, Strelec I, Moslavac T, Šubarić D, Pavić V and Molnar M 2021 Lipoxygenase Inhibition by Plant Extracts. *Biomolecules* 11(2): 152-164.

Maigida HH and Bukar BB 2024 Cardioprotective and antioxidant properties of the arils of the fruit of *Blighia sapida* KD koenig (Sapindaceae) in Isoprenaline-induced myocardial infarction in Wistar rats. *Afr. J. Pharma. Res. Dev.* 16(1): 1-13.

Matata DZ, Ngassapa OD, Machumi F and Moshi MJ 2018 Screening of plants used as traditional anticancer remedies in Mkuranga and same districts, Tanzania, using brine shrimp toxicity bioassay. *Evid. Based Complement Alternat. Med.* 2018(1): 3034612.

Mohammed RS, Abdulsalam MM and Kntapo FM 2023 Hypoglycemic Effect of *Anthocleista djalonensis* and *Blighia unijugata*. *Nig. J. Chem. Res.* 28(1): 025-039.

Moualek I, Aiche GI, Guechaoui NM, Lahcene S and Houali K 2016 Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract. *Asian Pac. J. Trop. Biomed.* 6(11): 937-944.

Muscolo A, Mariateresa O, Giulio T and Mariateresa R 2024 Oxidative Stress: The Role of Antioxidant Phytochemicals in the Prevention and Treatment of Diseases. *Int. J. Mol. Sci*. *25*(6): 3264.

Nabede A, Sina H, Souho T, Mélila M, Ouadja B, Salami HA, Assogba SA, Adjanohoun A, Baba-Moussa L and Amouzou KS 2025 Effects of *Blighia sapida* aril oil consumption on hemoglobin, serum proteins, and some vital organs in Wistar rats. *Sci. Afr.* 27: p.e02507.

Odekanyin OO, Aibinu TT and Abioye O 2017 Antibacterial and Hemagglutinating Activity of the Fruit Pulp of *Trilepisium madagascariense* DC. *Biotechnol. J. Int.* 18(2): 1-9.

Oderinde RA, Ajayi IA and Adewuyi A 2008 Evaluation of the mineral nutrients, characterization and some possible uses of *Blighia unijugata* baker seed and oil. *Seed Sci. Biotechnol.* 2(2): 79-82.

Offor CE, Onwe NJ, Agbafor KN and Nwangwu S 2014 Determination of Proximate and Vitamin Compositions of *Blighia unijugata* Leaves. *Acad. J. Nutr.* 3(3): 22-25.

Ojewole JAO 2005 Antinociceptive, anti-inflammatory of *Blighia unijugata* bark aqueous extract, *J Ethnopharmacol*. 99:13-19.

Okaiyeto K and Oguntibeju OO 2021 African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *Int. J. Environ. Res. Public Health 18*(11): 5988.

Oloyede GK, Onocha PA, Ikiroma TR and Olusola OW 2023 Variation in chemical composition, insecticidal and antioxidant activities of essential oils from the leaves, stem barks, and roots of *Blighia unijugata* (Baker) and *B. sapida* (KD Koenig). *Int. J. Plant Based Pharma.* 3(1): 10-17.

Osuala FN, Odoh UE, Onuigbo V and Ohadoma SC 2020 Pharmacognostic Screening and Anti-inflammatory Investigation of the Methanol extract of stem bark of *Blighia unijugata* Baker (Sapindaceae). *J. Drug Deliv. Ther.* 10(4): 146-152.

Rizvi NB, Aleem S, Khan MR, Ashraf S and Busquets R 2022 Quantitative estimation of protein in sprouts of *Vigna radiate* (Mung beans), *Lens culinaris* (Lentils), and *Cicer arietinum* (Chickpeas) by Kjeldahl and Lowry methods. *Molecules*, 27(3): 814.

Rubio CP, Hernández-Ruiz J, Martinez-Subiela S, Tvarijonaviciute A and Ceron JJ 2016 Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. *BMC Vet. Res.* 12: 1-7.

Ruch RJ, Crist KA and Klaunig JE 1989 Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. *Toxicol. Appl. Pharmacol.* 100(3): 451-464.

Sadiq H, Sadiq H, Sohail A, Basit A, Akhtar N, Batool K, Hisaindee S and Asghar L 2023 Assessment of antioxidant activity of pure graphene oxide (GO) and composite V_2O_5/GO using DPPH radical and H_2O_2 scavenging assays. *J. Sol-Gel Sci. Technol.* 108(3): 840-849.

Salami SA, Osukoya OA, Adewale OB, Odekanyin OO, Obafemi TO and Kuku A 2023 Bioactivities of *Garcinia kola* enzymatic hydrolysates at different enzyme–substrate ratios. *AMB Express* 13 (1): 78.

Vasudev A and Sohal SK 2019 Negative effect of non-host protease inhibitors partially purified from *Cassia glauca* seeds on developmental physiology of *Spodoptera litura. Int. J. Trop. Insect. Sci.* 39: 1-8.

Yesmin S, Paul A, Naz T, Rahman AA, Akhter SF, Wahed MII, Emran TB and Siddiqui SA 2020 Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*). *Clin Phytosci*. 6: 59-68.