Prev. Nutr. Food Sci. 2023;28(2):160-169
https://doi.org/10.3746/pnf.2023.28.2.160
ISSN 2287-8602

Functional Bioactivities of Soluble Seed Proteins from Two Leguminous Seeds

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ABSTRACT: Storage proteins from *Sphenostylis stenocarpa* and *Phaseolus lunatus* were fractionated, and their *in vitro* bioactivities were investigated. Albumin, globulin, prolamin, and glutelin constituents of the respective seeds were successively fractionated using the modified Osborne method. Phenylmethylsulfonyl fluoride (1 mM) was used as a protease inhibitor. The antioxidant, anti-inflammatory, and acetylcholinesterase-inhibitory activities of the protein fractions were evaluated using different appropriate techniques. Globulin was the predominant fraction, with a yield of 43.21±0.01% and 48.19±0.03% for *S. stenocarpa* and *P. lunatus*, respectively, whereas prolamin was not detected in both seeds. The protein fraction markedly scavenges hydroxyl radicals, nitric oxide radicals, and 2,2-diphenyl-1-picryldydrazyl radicals with concomitant high free radical-reducing power. Albumin and globulin fractions elicited the highest acetylcholinesterase-inhibitory potential of 48.75% and 49.75%, respectively, indicating their great application potential in managing neurodegenerative diseases. In this study, the albumin, globulin, and glutelin fractions of these underutilized legumes showed great analeptic bioactivities, which could be utilized as health-promoting dietary supplements/products.

Keywords: antioxidant, anti-inflammatory, bioactive proteins, neurodegenerative disease, underutilized legumes

INTRODUCTION

In addition to the generation of biosynthetic and energy-yielding compounds such as amino acids, which are essential in growth and development, dietary proteins can also serve as precursors of biologically active peptides (Wu, 2016; Yang et al., 2021). At present, the increasing demand for plant proteins and plant-based functional foods promotes research into diverse protein derivatives such as concentrates, hydrolysates, and isolates, thereby raising awareness into the exploration of nontraditional protein sources (Sun et al., 2017; Wang and Xiong, 2019; Ashaolu, 2020; Aschemann-Witzel et al., 2021). Furthermore, the low-cost production, high sustainability, and nutritional significance of these plant proteins have promoted their application in food processes (Rebello et al., 2014; Carbonaro et al., 2015; Ahnen et al., 2019).

Protein energy malnutrition and micronutrient deficiency contribute significantly to infant morbidity and mortality, affecting about 151 million children globally (FAO, 2018). About 30% of children in Africa and Asia suffer from protein malnutrition (Cheng et al., 2019). In developing countries, children and nursing mothers are also affected to a certain extent, and the Food and Agriculture Organization projected that approximately 822 million people might be affected by this menace globally because of the coronavirus disease 2019 pandemic (FAO, 2020). This finding indicates the need for alternative sources, such as plant proteins that are ubiquitous and affordable, although the demand for limited food to mitigate malnutrition and hunger is increasing (Runsewe-Abiodun et al., 2018; Bolarinwa et al., 2019; Jin et al., 2021). Furthermore, plant-based proteins and peptides can cause diverse health-promoting bioactivities in humans and animals, thereby enhancing research efforts into their isolation and fractionation (Ajibola et al., 2016; Iyenagbe et al., 2017; Ulloa et al., 2017; Aderinola et al., 2018). These bioactivities include antioxidant, anti-inflammatory, antimicrobial, and immune-modulating activities (Hernández-Ledesma et al., 2013; Liu, 2013; Majumder et al., 2016; Ribeiro et al., 2019; Capraro et al., 2021).

Seed storage proteins (SSPs) are produced in maturing endosperm and classified into water-soluble albumin, salt-soluble globulin, alcohol-soluble prolamin, and alka-

Received 5 July 2022; Revised 11 April 2023; Accepted 17 April 2023; Published online 30 June 2023
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line-soluble glutelin (Ciccocioppo et al., 2005; Kumar et al., 2019). SSPs serve as amino acids and an essential nutrient pool necessary for germination, which can be used to determine seed nutritional quality (Gallardo et al., 2007; Tan-Wilson and Wilson, 2012; Joshi et al., 2017; Gacek et al., 2018).

African yam bean (*Sphenostylis stenocarpa*) and lima bean (*Phaseolus lunatus*) are underutilized leguminous plants (Family: *Fabaceae*) moderately consumed in many parts of West Africa such as Nigeria, and the legumes are rich in protein (Maphosa and Jideani, 2017; George et al., 2020; Ojuederie et al., 2021). Most legumes in this category thrive in harsh conditions, and they are of high nutritional quality compared with common legumes. They are also considered as healthy alternatives to animal products (Kalidass et al., 2014; Cheng et al., 2019; Mabhaudhi et al., 2019). The overproduction of free radical and reactive species has been linked to the etiology of many human diseases (Wang et al., 2021). Hence, in this study, soluble proteins, such as albumin, globulin, and glutelin, were fractionated from the seeds of two underutilized legumes, namely, *S. stenocarpa* and *P. lunatus*. In addition, the antioxidative, anti-inflammatory, and acetylcholinesterase-inhibitory activities of such proteins were investigated to utilize their potential development as protein-based functional food products.

MATERIALS AND METHODS

Materials and reagents

S. stenocarpa and *P. lunatus* seeds were obtained from a farmland (Abagbooro village) in Ile-Ife, Southwest Nigeria, and identified at the Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Bovine serum albumin, Folin-Ciocalteau reagent, petroleum ether, acetone, Tris-hydrochloride, ethylenediaminetetraacetic acid (EDTA), phenylmethylsulfonyl fluoride (PMSF), 2,2-diphenyl-1-picryldydrazyl (DPPH), 2,4,6-tripyridyl-*S*-triazine, ferrozine, and deoxyribose were purchased from Sigma Chemical Company. Hydrochloric acid (HCl), methanol, gallic acid, sulfuric acid (H_2SO_4), sodium carbonate (Na_2CO_3), aluminum chloride, potassium acetate, potassium persulphate, sodium nitroprusside, hydrogen peroxide, glacial acetic acid, naphthylethylenediamine dichloride, nicotinamide adenine dinucleotide hydride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and L-ascorbic acid were all purchased from Merck. All other chemicals and reagents used were of analytical grade.

Protein extraction and fractionation

S. stenocarpa and *P. lunatus* seed protein fractions were prepared using the Osborne method as described by Tovar-Pérez et al. (2017) with modifications. In brief, respective dried seeds were milled into flour (with a particle size of ≤0.177 mm), defatted repeatedly using a Soxhlet apparatus with petroleum ether (boiling point 70～80°C), air dried in a fume hood, and stored at 4°C. Dry acetone powder of the defatted seed flour was prepared by successive extraction in 80% (v/v) and 70% (v/v) cold aqueous acetone and air dried. Acetone dry powder (AcDP) (1:10 w/v) was extracted successively with 10 mM Tris-HC1 buffer at pH 7.5 (containing 2 mM EDTA) for 5 h and continuously stirred. The supernatant was collected by centrifugation (10,000 *g*, 30 min) constituting the albumin fraction. Globulin, prolamin, and glutelin fractions were successively obtained from the collected residues by centrifugation and extraction in 0.5 M NaCl (containing 2 mM EDTA and 10 mM Tris-HCl buffer, pH 7.5), 70% (v/v) ethanol, and 0.1 M NaOH followed by centrifugation and filtration. The protein fractions were dialyzed against deionized water for 72 h at 4°C using a 3-kDa cut-off dialysis tubing with frequent changes. PMSF (1 mM) was added to the extraction solution to prevent proteolytic digestion. The resulting protein fractions were lyophilized and stored at −20°C. The protein concentration was estimated by using the method of Lowry et al. (1951).

Determination of antioxidant and anti-inflammatory activity

***Ferric reducing power assay*:** In this study, the assay method described by Shahi et al. (2020) was used with slight modifications. In general, aliquots (100 μg/mL) of the respective protein fractions (albumin, globulin, and glutelin) were dissolved in 0.2 mM phosphate buffer (0.5 mL; pH 6.6) and 0.5 mL of potassium ferricyanide (1% w/v). The resulting test solutions were kept undisturbed at 50°C for 20 min. TCA (10%, 2.5 mL) was introduced to the mixture and centrifuged at 700 *g* for 10 min. An aliquot (1 mL) of the obtained supernatant was mixed with 1 mL of $FeCl_3$ (0.1%, 0.5 mL), and the optical density was measured at 700 nm. Gallic acid, ascorbic acid, and Trolox (100 μg/mL) were used as positive standards. The high absorbance of the reaction mixture indicated the reductive potential of the protein fractions.

***DPPH radical-scavenging assay*:** The assay procedure of Wu et al. (2003) was used with slight modifications. In brief, aliquots (1 mL) of varying concentrations (50～2,000 μg/mL) of each protein fraction (albumin, globulin, and glutelin) were added to 1 mM DPPH (1 mL) in a methanol solution. The resulting mixture was vortexed, centrifuged (700 *g* for 10 min), and then left placidly in a pitch black cupboard for 30 min at room temperature. The absorbance of the supernatant was measured at 517 nm against the DPPH control containing 1 mL of methanol in place of protein fractions. Gallic acid, ascorbic acid, and Trolox were used as positive standards. The proportion of

DPPH radical inhibition was estimated using the following formula:

$$\% \text{ Inhibition} = \frac{A_{blank} - A_{sample}}{A_{control}} \times 100$$

where A_{blank} was the absorbance of the blank, and A_{sample} was the absorbance of the sample.

***Hydroxyl radical-scavenging assay*:** The hydroxyl radical-scavenging activity of protein fractions was carried out in accordance with the assay protocol of Chung et al. (1997) as described by Shahi et al. (2020) with slight modifications. In general, 0.1 mL of 10 mM EDTA, 0.01 mL of 10 mM $FeCl_3$, 0.1 mL of 10 mM H_2O_2, 0.36 mL of 10 mM deoxyribose, 1.0 mL of respective protein fractions (albumin, globulin, and glutelin; 10~1,000 μg/mL), 0.33 mL of phosphate buffer (50 mM, pH 7.4), and 0.1 mL of ascorbic acid were added sequentially. After incubating for 1 h at 37°C, an aliquot (1.0 mL) of 5% TCA and 1.0 mL of 0.5% TBA were added. Absorbance was measured at 532 nm. Trolox, gallic acid, and ascorbic acid were used as the standard. The percentage of scavenged hydroxyl radicals was estimated using the following equation:

$$\% \text{ Inhibition} = \frac{1 - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control at 532 nm, and A_{sample} is the absorbance of sample solutions.

***Scavenging of hydrogen peroxide*:** The ability of the protein fractions to scavenge hydrogen peroxide was determined in accordance with the method of Nabavi et al. (2008; 2009). A spectrophotometer was used to estimate the quantity of hydrogen peroxide by measuring the absorbance at 230 nm. In distilled water, protein fractions (0.1~1.0 mg/mL) were mixed with a hydrogen peroxide solution (0.6 mL, 40 mM). After 10 min, the absorbance of hydrogen peroxide at 230 nm was measured against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenged by the protein fractions and reference chemicals was calculated using the following formula:

$$\% \text{ Scavenged hydrogen peroxide} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance in the presence of the sample of protein fractions and standards.

***Nitric oxide (NO) radical-scavenging assay*:** The NO radical-scavenging activity of the protein fractions was determined as described by Ebrahimzadeh et al. (2009). An aliquot (1 mL) of sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.2) was mixed with varying concentrations of each protein fraction (albumin, globulin, and glutelin) and distilled water. The resulting mixture was kept at 25°C for 150 min, and the Griess reagent (0.5 mL) was added. The generated pink chromophore absorbance was measured at 546 nm. Trolox, gallic acid, and ascorbic acid were used as positive controls. All experiments were conducted in three determinations. The percentage inhibition was determined using the following formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample.

***Lipid peroxidation inhibition (LPI) assay*:** The LPI assay was performed using the method of Liu et al. (2013) with slight modifications. The excised rat liver was coalesced in phosphate buffer (pH 7.4) and centrifuged to obtain liposomes. An aliquot (0.5 mL) of supernatant, 10 mM $FeSO_4$ (100 μL), 0.1 mM ascorbic acid (100 μL), and 0.3 mL of different protein fractions (albumin, globulin, and glutelin) or standards at varied concentrations were combined. The resulting solution was kept placid at 37°C for 20 min. After boiling, an aliquot of TCA (1 mL, 2%) and TBA (1.5 mL, 1%) was added. Finally, the reaction mixture was reheated for 15 min at 100°C before cooling to room temperature. The absorbance was measured at 532 nm after cooling. The percentage LPI was determined using the following equation:

$$\% \text{ LPI} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the extractives (soluble proteins) or standards. Afterward, the percentage of inhibition was plotted against the concentration.

***Acetylcholinesterase-inhibitory assay*:** The acetylcholinesterase-inhibitory activity of the protein fractions (albumin, globulin, and glutelin) was investigated using the method of Ellman et al. (1961) with slight modifications. Aliquots of rat brain homogenate were used to measure the acetylcholinesterase activity, a marker for cholinergic neurotransmission. In general, the acetylcholinesterase-inhibitory activity of the protein fractions in the brain homogenate was measured by adding an aliquot (2.6 mL) of phosphate buffer (0.1 M, pH 7.4), 0.8 mL of the respective protein fractions, 0.1 mL of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB), and 0.4 mL of the homogenate. Then, the reaction mixture was spiked with 0.1 mL of acetylthiocholine iodide solution. The absorbance was measured at 412 nm, and the change in absorbance was recorded every 2 min for 10 min. The increase in color

produced when thiocholine reacts with DTNB was used to determine the rate of acetylcholinesterase activity. The rate of acetylcholinesterase activity was computed and represented as moles/min/g tissue.

$$\text{Rate} = \frac{\Delta\text{Absorbance}}{\text{min}}$$

$$\text{Enzymatic activity} = \%\text{Rate}$$

***Half-maximal inhibitory concentration (IC_{50}) value*:** IC_{50} of each biological activity is calculated using the linear regression equation of the graphs, and the logarithm-transformed x-axis was used for non-linear activity (https://www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm).

Statistical analysis

Data obtained during the experiment were presented as mean±standard error and subjected to one-way analysis of variance. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Protein isolation and fractionation

In addition to protein concentrates and isolates, the investigation of soluble seed protein fractions as potential vegetal sources of health-promoting ingredients has become an emerging trend globally (Aderinola et al., 2018; Dey and Sinhababu, 2018; Nwachukwu and Aluko, 2018). In this study, globulin was the predominant protein fraction of *S. stenocarpa* and *P. lunatus* seeds, with a respective yield of 43.21±0.01% and 48.19±0.03%, respectively, followed by albumin and glutelin (Table 1). Prolamin was not detected in both seeds. The respective seeds showed high globulin and albumin yields as they have been documented to be quite substantial in plant seeds, particularly those belonging to the legume family (Rodríguez-Ambriz et al., 2005; Gulewicz et al., 2008; Boye et al., 2010). A previous study conducted by Ajibola et al. (2016) reported a 91% globulin yield in *S. stenocarpa*, whereas a 62.3% albumin yield was reported for *P. lunatus* seeds (Gallegos Tintoré et al., 2004). Elevated globulin yields have also been reported in *Sesame indica* and *Citrullus lanatus* seeds (Dash and Ghosh, 2017; Idowu et al., 2021), whereas albumin was the predominant fraction obtained from *Moringa oleifera* seeds (Aderinola et al., 2018). The yield of soluble seed protein fractions slightly varies as they can result from a range of factors from fractionation methods, storage conditions, and ecological distribution of the seeds (Tovar-Pérez et al., 2017; Zambrano et al., 2020).

Bioactivities of protein fractions

***Ferric reducing power and hydroxyl radical inhibitory activity*:** Protein isolates with diverse activities can be obtained by controlling the protein solubility, which exploits their hydrodynamic attributes (Klupšaitė and Juodeikienė, 2015). The result shown in Fig. 1A indicates the ferric reducing power of varying concentrations of albumin, globulin, and glutelin fractions in *S. stenocarpa* and *P. lunatus* relative to standard antioxidants (Trolox, ascorbic acid, and gallic acid). The ferric reducing power of the soluble proteins was evaluated by monitoring the change in coloration of the testing mixture from yellow to green depending on the reducing power of the test specimen. These protein fractions showed significant ($P<0.05$) ferric radical-reducing power at the tested concentrations. Notably, the *P. lunatus* globulin fraction exhibited the highest ferric reducing power activity at 2,000 μg/mL. The Fe^{3+}/ferric cyanide complex is reduced to ferrous form in the sample solution because of the presence of reducing agents. Consequently, the ferrous ion can be observed by measuring the absorbance at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability. A similar result has been reported for the globulin fraction of *C. lanatus* as the reducing properties of protein have been associated with inherent hydrophobic amino acids (Zhang et al., 2008; Dash and Ghosh, 2017).

The results shown in Fig. 1B indicated that at 2,000

Table 1. Protein content obtained from *Sphenostylis stenocarpa* and *Phaseolus lunatus* seeds

Protein fractions	*Sphenostylis stenocarpa*		*Phaseolus lunatus*	
	Amount present (mg/g AcDP)	Protein yield (%)	Amount present (mg/g AcDP)	Protein yield (%)
AcDP flour	20.00±0.01	100.00	20.00±0.01	100.00
Albumin	1.38±0.03^{b}	37.50±0.02^{b}	1.12±0.01^{b}	36.97±0.01^{b}
Globulin	1.59±0.02^{c}	43.21±0.01^{c}	1.46±0.02^{c}	48.19±0.03^{c}
Prolamin	ND	ND	ND	ND
Glutelin	0.71±0.09^{a}	19.29±0.02^{a}	0.45±0.11^{a}	14.14±0.01^{a}

Values are presented as mean±SD.
Means with different letters (a-c) are significantly different at $P<0.05$.
AcDP, acetone dry powder; ND, not detected.

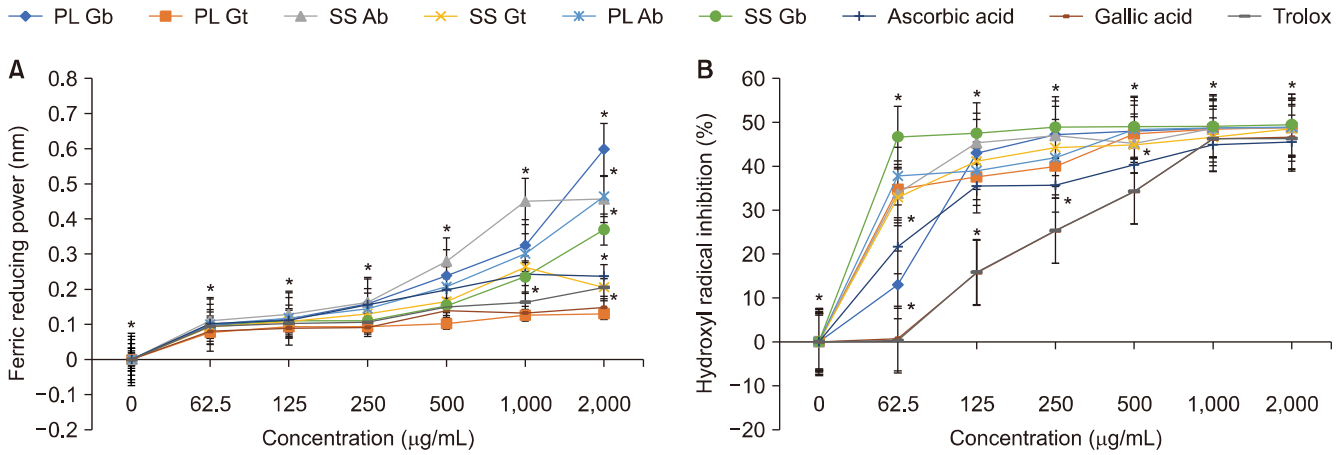

Fig. 1. Ferric reducing power (A) and hydroxyl radical inhibitory activity (B) of albumin, globulin, and glutelin fractions of *Sphenostylis sternocarpa* and *Phaseolus lunatus* seeds. PL Gb, *P. lunatus* globulin; PL Gt, *P. lunatus* glutelin; SS Ab, *S. stenocarpa* albumin; SS Gt, *S. stenocarpa* glutelin; PL Ab, *P. lunatus* albumin; SS Gb, *S. stenocarpa* globulin. Data were presented as mean±standard deviation and differences considered statistically significant at $^{*}P<0.05$.

g/mL, a significant difference ($P<0.05$) in hydroxyl radical-scavenging activities of albumin, globulin, and glutelin fractions of *S. stenocarpa* and *P. lunatus* was observed relative to the positive standards. At all tested concentrations, these protein fractions exhibited a higher hydroxyl radical-scavenging ability than the standards, indicating their potent antioxidant properties and putative chemotherapeutic potentials. Free radicals have mutagenic and carcinogenic qualities because of their unique association with intracellular macromolecules, which are essential steps in the carcinogenesis pathway (cancer formation). Hydroxyl radicals that are produced during biotransformation reactions in the body can induce harm to every living cell; superoxide dismutase transforms superoxide radicals to hydrogen peroxide, which can produce reactive radicals when ions of specific divalent metals are present (Thorpe et al., 2013). Aromatic amino acids, such as tyrosine, have been associated with the hydroxyl radical sequestration ability of proteins (Ajibola et al., 2011; Yang et al., 2018). Similar OH radical-scavenging results have been reported for the sweet potato protein (Zhang et al., 2012). In the current study, *P. lunatus* globulin showed the best hydroxyl radical-scavenging activity with the lowest IC_{50} value compared with other storage proteins and the standards used (Table 2).

***Hydrogen peroxide and DPPH radical-inhibitory activity*:** Hydrogen peroxide is a mild oxidizing agent that can be dangerous when degraded into oxygen and water, producing hydroxyl radicals that can damage DNA and trigger lipid peroxidation (Oladele et al., 2020b). Some biomolecules can be inhibited by hydrogen peroxide by oxidizing their crucial thiol (-SH) groups. The ability of plant products to donate hydrogen is an index used to determine the antioxidant capability of plant products (Oyeleke et al., 2022). At the tested concentrations, albumin, globulin, and glutelin fractions of *S. stenocarpa* and *P. lunatus* seeds significantly ($P<0.05$) scavenged hydrogen peroxide similar to the standard antioxidants used (Fig. 2A). This biological activity may be attributed to the presence of unique inherent amino acids in these protein fractions,

Table 2. Half-maximal inhibitory concentration of biological activities of storage proteins (unit: μg/mL)

	Nitric oxide	Hydrogen peroxide	Total antioxidant	Lipid peroxidation inhibition	DPPH	Hydroxyl radical	AChE
Gallic acid	441.97	2,289.27	ND	75.34	1,589.08	1,765.84	ND
Trolox	511.35	4,253.59	ND	474.56	1,730.67	1,775.15	ND
Ascorbic acid	439.31	3,018.25	ND	716.29	2,231.70	2,101.14	ND
PL Ab	7,296.56	2,047.18	2,072.28	−330.78	10,370.26	1,741.67	2,458.57
PL Gt	3,133.14	3,557.03	2,125.75	1,038.15	2,597.97	1,742.12	2,038.85
PL Gb	2,496.77	3,606.22	2,379.70	635.02	2,925.06	1,597.47	2,139.58
SS Ab	6,414.74	3,779.06	1,900.18	−1,512.86	3,759.02	1,838.18	1,846.47
SS Gt	5,708.83	5,149.68	1,783.45	146.88	2,790.79	1,947.41	2,100.80
SS Gb	4,245.74	1,996.31	1,789.10	−4,699.65	9,428.18	2,242.00	1,971.11

PL Ab, *Phaseolus lunatus* albumin; PL Gt, *P. lunatus* glutelin; PL Gb, *P. lunatus* globulin; SS Ab, *Sphenostylis stenocarpa* albumin; SS Gt, *S. stenocarpa* glutelin; SS Gb, *S. stenocarpa* globulin; DPPH, 2,2-diphenyl-1-picryldydrazyl; AChE, acetylcholinesterase; ND, not detected.

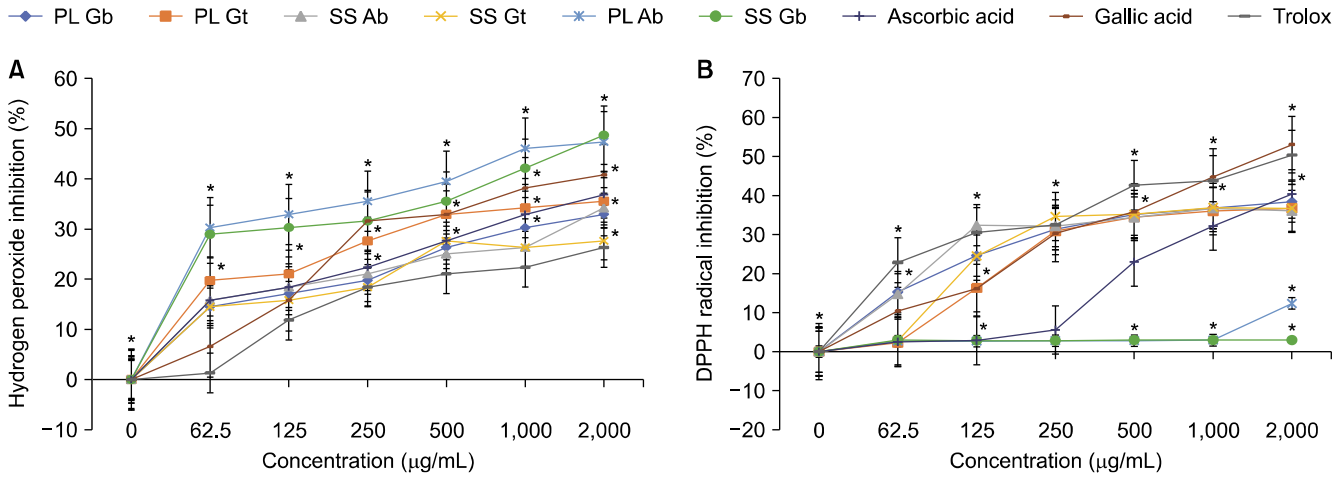

Fig. 2. Hydrogen peroxide inhibitory activity (A) and 2,2-diphenyl-1-picryldydrazyl (DPPH) radical inhibition activity (B) of albumin, globulin, and glutelin fractions of *Sphenostylis sternocarpa* and *Phaseolus lunatus* seeds. PL Gb, *P. lunatus* globulin; PL Gt, *P. lunatus* glutelin; SS Ab, *S. stenocarpa* albumin; SS Gt, *S. stenocarpa* glutelin; PL Ab, *P. lunatus* albumin; SS Gb, *S. stenocarpa* globulin. Data were presented as mean±standard deviation and differences considered statistically significant at $^{*}P<0.05$.

particularly charged amino acids, which can neutralize this radical via electron transfer. Cysteine and methionine residues of proteins have also been reported to react directly with peroxides (Zhou and Elias, 2014). Compared with other storage proteins and standards used in this study, *S. stenocarpa* globulin demonstrated the greatest hydrogen peroxide-scavenging activity with the lowest IC_{50} value of 1,996.31 μg/mL (Table 2).

The result shown in Fig. 2B indicates that albumin, globulin, and glutelin fractions of *S. stenocarpa* and *P. lunatus* significantly ($P<0.05$) scavenged DPPH radical as compared with gallic acid, Trolox, and ascorbic acid. At 250 μg/mL, the glutelin fraction exhibited the highest DPPH radical-scavenging activity. Nevertheless, at 1,000 μg/mL, almost all soluble proteins displayed a higher DPPH radical-scavenging activity than ascorbic acid. Labile hydrogen can be abstracted by DPPH, and its capacity to neutralize the DPPH radical is related to the prevention of lipid peroxidation (Kedare and Singh, 2011). Thus, the DPPH radical-scavenging activity of these soluble proteins indicates their antioxidant capacity. DPPH-radical-scavenging activity has also been reported in proteins isolated from *Bunium persicum* (Shahi et al., 2020). The radical-scavenging activity of protein molecules is related to a significant proportion of hydrophobic amino acids primarily because of the chemistry of aromatic and sulfur-containing residues (Zou et al., 2016; Yang et al., 2018). In addition, the DPPH radical-scavenging activity has been reported in proteins isolated from *Solanum tuberosum* (Liu et al., 2013).

***NO radical-scavenging and lipid peroxidation inhibitory activities*:** A significant increase ($P<0.05$) in the NO-inhibitory activity of the fractionated *S. stenocarpa* and *P. lunatus* seed protein was observed (Fig. 3A). Although none of

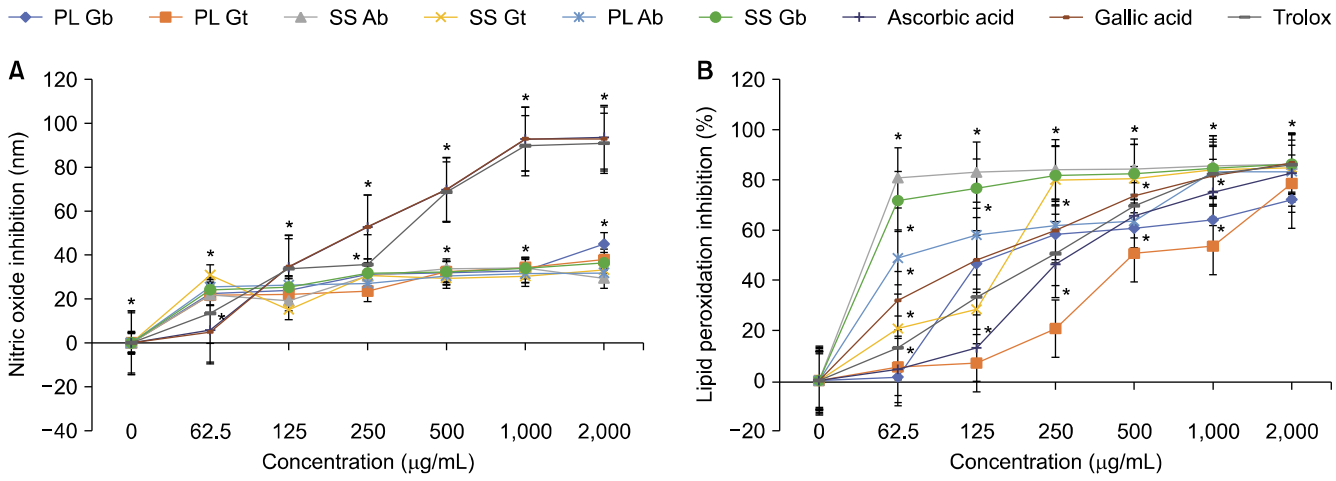

Fig. 3. Nitric oxide inhibitory activity (A) and lipid peroxidation inhibitory activity (B) of albumin, globulin, and glutelin fractions of *Sphenostylis sternocarpa* and *Phaseolus lunatus* seeds. PL Gb, *P. lunatus* globulin; PL Gt, *P. lunatus* glutelin; SS Ab, *S. stenocarpa* albumin; SS Gt, *S. stenocarpa* glutelin; PL Ab, *P. lunatus* albumin; SS Gb, *S. stenocarpa* globulin. Data were presented as mean±standard deviation and differences considered statistically significant at $^{*}P<0.05$.

the fractionated proteins has a NO radical-scavenging activity up to any of the standard antioxidants used, their activities are quite pronounced. This finding indicates the potential anti-inflammatory potential of the proteins. NO mediates nitrosative stress and serves as an inflammatory biomarker associated with the pathophysiology of many diseases. NO cannot initiate DNA injury alone but in combination with oxygen, which yields dinitrogen trioxide, and the reaction of NO with superoxide results in the formation of peroxynitrite (Thorsteinsdottir et al., 2011). These molecules are highly reactive, and NO production can aggravate inflammatory conditions. NO is primarily synthesized via inducible NO synthase under inflammatory conditions. *P. lunatus* globulin exhibited the best NO radical-scavenging activity with the lowest IC_{50} value compared with other storage proteins used in the study (Table 2).

Our findings indicated that *S. stenocarpa* albumin displayed the highest lipid peroxidation inhibitory activity at 62.5, 125, 250, and 500 μg/mL (Fig. 3B). However, at 2,000 μg/mL, all proteins exhibited significant ($P<0.05$) lipid peroxidation inhibitory activities compared with the standard antioxidants used probably because of the presence of phenylalanine, leucine, and tyrosine residues as these amino acids facilitate protein solubility, thereby allowing interaction with radical species (Asoodeh et al., 2016).

***Total antioxidant capacity and acetylcholinesterase-inhibitory activity*:** At 2,000 μg/mL, all the respective protein fractions, including albumin, globulin, and glutelin, displayed a high total antioxidant capacity (Fig. 4A), which indicates that they could be good sources of antioxidants. The oxidative macromolecular damage initiated by free radicals and reactive oxygen species (ROS)/reactive nitrogen species (RNS) stress includes DNA oxidation, glycoxidation, and protein oxidation, which have been reported to play a vital role in the development of many diseases such as diabetes, neurodegenerative disorders, cardiovascular diseases, and cancer (Nunomura et al., 2006; Halliwell and Gutteridge, 2015; Chen et al., 2021). Oxidative stress is defined as an imbalance between the synthesis of ROS/RNS and antioxidants in favor of the former. Oxidative stress is induced by free radicals such as hydroxyl radicals, alkoxyl radicals, and peroxyl radicals, among others, which tend to stabilize in healthy human cells by electron pairing with biological molecules such as DNA, proteins, and lipids. This concept has led to the assertion that oxidative stress plays a pivotal role in the pathogenesis of numerous diseases (Oladele et al., 2017, 2019, 2020a). Nevertheless, *P. lunatus* albumin and *S. stenocarpa* glutelin displayed the highest total antioxidant capacity and the lowest IC_{50} value compared with other storage proteins used in this study (Table 2).

The protein fractions elicited high acetylcholinesterase-inhibitory activity (Fig. 4B), indicating their potential application in the management of neurodegenerative diseases. Acetylcholine is a neurotransmitter in the cholinergic system, which plays a key role in central and peripheral nervous system functions, such as learning, memory, movement, control, and cerebral blood flow modulation, making it crucial for neurological functions. Acetylcholinesterase, an enzyme that hydrolyzes the neurotransmitter, regulates acetylcholine levels (Kiehn, 2006; Rösser et al., 2008). Inhibiting the activity of acetylcholinesterase enhances the treatment of neurodegenerative diseases via the upregulation of acetylcholine. In this study, the albumin, globulin, and glutelin fractions of *S. stenocarpa* and *P. lunatus* showed moderate inhibitory effects against acetylcholinesterase, which indicate their beneficial effects in managing neurodegenerative diseases, when com-

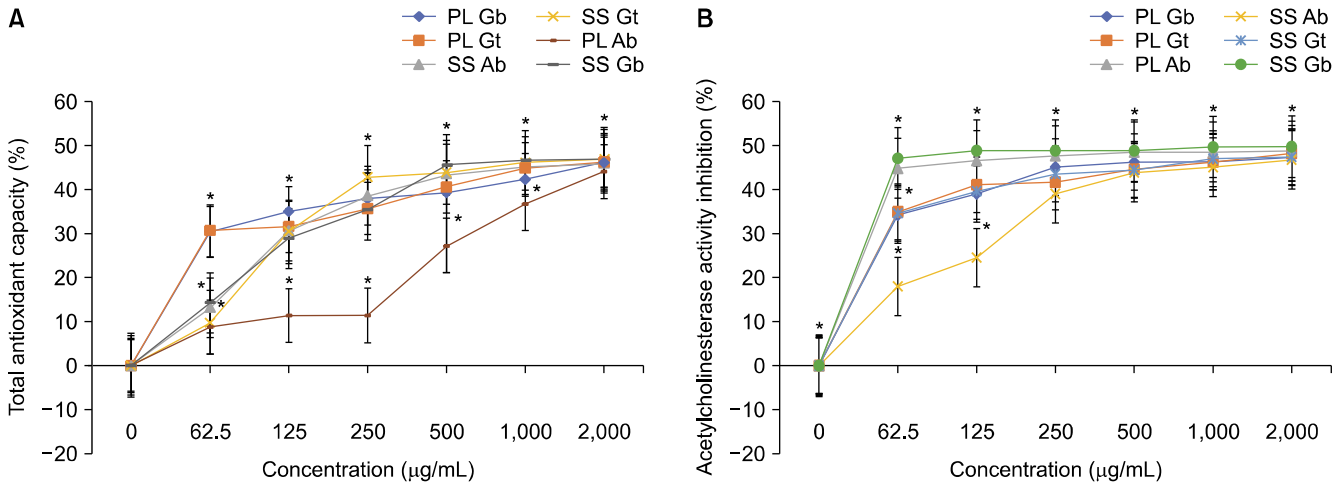

Fig. 4. Total antioxidant capacity (A) and acetylcholinesterase activity (B) of albumin, globulin, and glutelin fractions of *Sphenostylis sternocarpa* and *Phaseolus lunatus* seeds. PL Gb, *P. lunatus* globulin; PL Gt, *P. lunatus* glutelin; SS Ab, *S. stenocarpa* albumin; SS Gt, *S. stenocarpa* glutelin; PL Ab, *P. lunatus* albumin; SS Gb, *S. stenocarpa* globulin. Data were presented as mean±standard deviation and differences considered statistically significant at $^*P<0.05$.

pared with other storage proteins. In addition, *S. stenocarpa* albumin demonstrated the best *in vitro* acetylcholinesterase-inhibitory activity with the lowest IC_{50} value of 1,846.47 μg/mL (Table 2). In this study, the functional bioactivities reported for the albumin, globulin, and glutelin fractions of *P. lunatus* and *S. stenocarpa* supported the recent study of Okagu and Udenigwe (2022), that is, these soluble protein fractions in legumes can be exploited as potential candidates for health-promoting therapeutic products.

SSPs are multimeric proteins implicated in supplying the dietary protein requirements of more than half of the world population (Galvez et al., 2009; Kawakatsu and Takaiwa, 2010; Narvariya et al., 2017). Studies have shown that food or seed proteins play a profound role in health benefits by improving many chronic diseases via modulation and interaction with specific biochemical markers, thereby supporting the findings in this study. Therefore, *S. stenocarpa* and *P. lunatus* seeds are rich sources of soluble proteins with remarkable health-promoting bioactivities as underutilized plants are known to be reservoirs for bioactive/functional compounds. These properties could improve their respective economic value and their development as functional complementary food products and/or protein-based nutraceuticals to manage specific diseases. However, further studies must be conducted on the purification, characterization, and structure-function relationship of these proteins toward full exploitation.

FUNDING

None.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflicts of interest

AUTHORS CONTRIBUTIONS

Concept and design: TSA, AK. Analysis and interpretation: TSA, BBD, JOO. Data collection: TSA, BBD, JOO, OMO. Writing the article: TSA, JOO. Critical revision of the article: TSA, JOO, AK. Final approval of the article: all authors. Statistical analysis: TSA, JOO. Overall responsibility: TSA, JOO, AK.

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