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Research Article

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Some Biological Activities of Ethanol Extract of Amaranthus lividus L. Seeds and Isolation of the Lectin from the Crude Extract

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Abstract

Background and Aims: Amaranth is a gluten-free pseudocereal with nutraceutical properties. The aim of this study was to evaluate the antioxidant, anti-cholinergic and cyclooxygenase (COX) inhibitory effects of the ethanol extract and isolate the lectin from the crude extract prepared from seeds of *Amaranthus lividus* L.

Methods: The dried powdered seeds were extracted with ethanol using a Soxhlet apparatus.

The antioxidant activity of the ethanol extract was estimated by DPPH, ABTS and superoxide radical scavenging activities, as well as the Trolox equivalent antioxidant capacity (TEAC) and Ferric-Reducing Antioxidant Power (FRAP). The total phenolic compound content was determined using the Folin-Ciocalteu reagent. The extract was screened for its acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities using Ellman's method. The ability of the extracts to inhibit COX-1 and COX-2 was determined by enzyme immunoassay. The lectin was purified from the crude extract by a two-step procedure of 50% ammonium sulphate precipitation and affinity chromatography on CNBr-activated Sepharose 4B, using ovalbumin as a ligand. The molecular weight was determined using SDS-PAGE.

Results: Results showed that the extract possesses *in* vitro antioxidant, AChE, BuChE, COX-1 and COX-2 inhibitory activities, suggesting that it may provide treatment options for oxidative stress mediated diseases like Alzheimer's, inflammatory and cancer diseases. The crude extract of the *A. lividus* seeds was found to contain lectin, evident by hemagglutination. The lectin was purified to homogeneity approximately 16-fold over the crude extract. It was determined to be a homodimer, and the molecular weight of the monomer corresponded to 34 kDa.

Conclusion: The results showed that the seeds of *A. lividus* contain valuable ingredients such as phenolic compounds and lectin that are beneficial for health.

Keywords *Amaranthus lividus* · Seeds · Antioxidant · Anti-inflammatory · Anti-cholinergic · Affinity chromatography · Lectin

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INTRODUCTION

Amaranthus species, widely used as grain and leaf vegetables around the world, have been recognised for their nutritious and therapeutic properties for the treatment/prevention of chronic diseases. Previous studies have shown that *Amaranthus* species have protective and curative properties such as protecting against oxidative stress-induced disorders, neutralising their degenerative consequences due to their strong antioxidant and free radical scavenging activities (Peter & Gandhi, 2017; Jimoh et al., 2022).

Amaranth seed is a gluten-free, highly nutritive pseudocereal with antioxidant, anti-allergic, anticancer, anti-inflammatory and hypocholesterolemic properties and is a food for patients with coeliac disease and immunodeficiencies (Yi, Kang & Bu, 2017; Caselato-Sousa & Amaya-Farfán, 2012). Amaranth grains are valued for their amino acid composition, which is superior to that of traditional grains. Amaranth seeds also contain significant amounts of mineral salts, vitamins, oil rich in unsaturated fatty acids, squalene, polyphenols, anthocyanins and flavonoids, tocopherols and tocotrienol (Peter & Gandhi, 2017; Paśko et al., 2009). The seeds of various species such as *A. caudatus* (Karamać et al., 2019; Aguilar-Felices et al., 2019; Peiretti et al., 2017; Li et al., 2015; Repo-Carrasco-Valencia et al., 2010; Alvarez-Jubete et al., 2010; Conforti et al., 2005), *A. cruentus* (Li et al., 2015; Paśko et al., 2009; Nsimba et al., 2008), *A. hypochondriacus* (Li et al., 2015; Iqbal et al., 2012; Lopez et al., 2011; Nsimba et al., 2008) and *A. viridis* (Lopez-Mejía et al., 2014; Ahmed et al., 2013) have been studied for their antioxidant activities. Among vegetable crops amaranth has been recognised as "having one of the top five antioxidant capacities" (Iqbal et al., 2012). Some therapeutic activities of Amaranth seeds such as antiproliferative, antihypertensive, antioxidant, and hypocholesterolemic effects have been attributed to the bioactive peptides like lunasin and lectin (Caselato-Sousa & Amaya-Farfán, 2012). Due to their reversible and specific carbohydrate or glycoconjugate binding specificity, lectins have become a beneficial tool for the structural investigation of carbohydrates on cell surfaces, isolation and characterisation of glycoproteins, in the diagnosis and treatment of malignant tumours (Sharon & Lis, 2004). Amaranth lectins have been isolated and purified from *A. caudatus* (Rinderle & Goldstein, 1989; Pardoe et al., 1970), *A. cruentus* (Koeppe & Rupnow, 1988), *A. leucocarpus* (Zenteno & Ochoa, 1988), *Amaranthus leucocarpus* syn. *hypocondriacus* (Hernández et al. 1999) seeds. Amaranth lectin from *A. caudatus* is a tumour factor (TF)-binding lectin, which may be used in the diagnosis of cancer a marker of the proliferation of malignant cells (Yu et al., 2001).

This study is expected to complement our previous studies on *Amaranthus lividus* species, reporting *A. lividus* vegetable as a natural antioxidant source (Ozden-Yilmaz et al., 2016; Ozden-Yilmaz et al., 2015; Ozsoy et al., 2009) by evaluating the antioxidant, anti-cholinergic and cyclooxygenase (COX) inhibitory effects of *A. lividus* seeds, the popular cultivar in Türkiye. Pursuing research on separation and characterisation of bioactive compounds having therapeutic properties, it was also aimed to purify the lectin from the seeds of the *A. lividus* to homogeneity by affinity chromatography on CNBr-activated Sepharose 4B and determine the sugar specificity and molar mass of the purified lectin.

MATERIALS AND METHODS

Seed Material and Preparation of The Extract

Specimens of *Amaranthus lividus* L. seeds were collected from Bartin Province of Türkiye and identified by Prof. Dr. Şukran Kültür, Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University. The dried powdered seeds (40 g) were extracted with 200 mL ethanol for 24 h in a Soxhlet apparatus. The ethanolic extract was evaporated to dryness at 40°C using a rotary evaporator, stored at –20°C and redissolved in ethanol before use.

Determination of The Total Soluble Phenolic Compounds, Antioxidant and AChE, BChE, COX-1 And COX-2 İnhibitory Activities

The total phenolic content of the extract was analysed using Folin-Ciocalteu's reagent (Slinkard & Singleton, 1977). The antioxidant activity was estimated by the thiobarbituric acid (TBA) method (Buege & Aust, 1978) based on the lipid peroxidation (LPO) of liposomes (Duh et al., 1999), Trolox Equivalent Antioxidant Coefficient (TEAC) (Re et al., 1999), Ferric ion Reducing Antioxidant Power (FRAP) (Benzie & Strain, 1996) 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\cdot}$) free (Brand-Williams et al., 1995) and superoxide anion radical scavenging activities assays (Aruoma et al., 1989). The methods were described in detail in our previous study on the antioxidant activity of *A. lividus* vegetable (Ozsoy et al., 2009).

The extract (5; 2.5; 1.25; 0.625 and 0.31 mg/mL) was screened for its acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities using Ellman's method (Ellman et al., 1961) with some modifications. Ellman assay solution was prepared by mixing 100 mM sodium phosphate buffer (pH 7.8), 75 mM/7.5 mM of acetylthiocholineiodide/butyrylthiocholineiodide and 10 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) to give a final concentration of 75 mM, 1/.01 mM and 0.3 mM, respectively. Galantamine (0.1; 0.05; 0.025; 0.0125 and 0.0625 mg/mL) was used as the standard drug in the tests. After 10 min pre-incubation of 5 µL of different concentrations of the extract and 5 µL of 1U of AChE or 5U of BuChE enzyme solution, the reaction was started by adding of 190 µL Ellman assay solution. The change in absorbance at 412 nm was measured for 10 min using a microplate reader (Biotek, Winooski, VT, USA). In parallel, the enzyme and substrate control experiments were performed using the same amount of distilled water instead of the enzyme and substrate solutions. The experiments were performed in triplicate. The results were expressed as EC_{50} values.

The cyclooxygenase inhibitory assay was performed using an enzyme immunoassay (EIA) kit (Cayman, USA). The manufacturer' s protocol was followed.

Statistical Analysis

The results were analysed using Graphpad Prism 9.0 (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) was performed for the evaluation of statistical differences. All data values are expressed as the mean ± SD. Results were considered statistically significant at $p < 0.05$.

Purification of Lectin From *A. lividus* Seeds By Affinity Chromatography

Thirty grams seeds were ground to a fine powder, then suspended in phosphate-buffered saline (PBS) and stirred for 2 hours at 4°C. The clear supernatant obtained by centrifugation of the homogenate at 13,000 rpm for 30 min was named the *A. lividus* seeds crude extract.

(1) Ammonium Sulphate (NH_4SO_4) Precipitation

The 50% NH_4SO_4 fraction was obtained by the precipitation of the soluble proteins in the crude extract by the addition of NH_4SO_4 to 50 % saturation and centrifugation at 13,000 rpm for 15 min at 4°C. The precipitate obtained was resuspended in PBS and after centrifugation to remove the insoluble material was dialysed against the same buffer and then applied to affinity chromatography.

(2) Affinity Chromatography On CNBr-Activated Sepharose 4B

Affinity chromatographic separation of the lectin was performed using ovalbumin as a ligand. The 50% $(NH_4)_2SO_4$ fraction was loaded on a column of ovalbumin bound-CNBr-activated Sepharose 4B. After washing with PBS, the ovalbumin-bound material was eluted with 1 M Tris-HCl (pH 7.4). The fractions with the protein content seen as a single peak at 280 nm were examined for hemagglutinating activity, dialyzed against PBS and concentrated by ultrafiltration.

Hemagglutination Test

The test was performed using a 4% human red blood cell group A suspension in PBS according to the serial 2-fold dilution procedure of samples using U-shape microplates (Zenteno & Ochoa, 1988). The results were expressed as a haemagglutinating unit (HU), which is the minimum amount of sample capable of inducing agglutination. Specific activity was considered to be the number of hemagglutinating units per mg of protein.

Hemagglutination Inhibition Test

Inhibition of *A. lividus* seeds lectin-induced hemagglutination was performed in a manner analogous to the hemagglutination test using various carbohydrates (D-lactose, D-mannose, D-fucose, methyl-α-D-mannopyranoside, D-glucosamine hydrochloride, D-raffinose, D-galactose, maltose) and a glycoprotein (ovalbumin) solutions in PBS at different concentrations (0.78-100 mM or mg/mL for ovalbumin) as follows: 25 µL of sugar samples in PBS were mixed with an equal volume of a lectin solution with four hemagglutination units. After standing for 30 min at room temperature and addition of 25 µL of a 4% erythrocyte suspension, the the sugar-binding specificity of the lectin was determined.

Protein Determination

The protein concentrations of the crude extract, 50% NH_4SO_4 fraction, eluates, and purified lectin were determined using the bicinchoninic acid (BCA) assay (Sigma-Aldrich; Merck KGaA). Five dilutions of the protein standard (bovine serum albumin) (1.6 mg/mL), which covered the linear range from 0.8 to 0.1 mg/mL were prepared. 10 µL of each standard or sample solution was pipetted into separate microplate wells. 80 µL of BCA working reagent was subsequently added to each well. The plate was incubated in the dark for 15 min at 60°C. The absorbance was read against the blank at 562 nm using a multiplate ELISA reader (Biotek Instruments, Inc.).

Polyacrylamide Gel Electrophoresis (PAGE)

The purity and molecular weight determination of the lectin was carried out by natural and sodium dodecyl sulphate (SDS)-PAGE according to Laemmli method (Laemmli, 1970), and visualised by 0.2 % Coomassie blue R-250 staining. The molecular weight was determined by comparison of the electrophoretic mobility of lectin with that of the marker proteins from Invitrogen (LC5615).

RESULTS

Extract Yield (Amount of Total Extractable Compounds) and Contents of The Total Phenolics

The ethanol extract of *A. lividus* prepared from 40 g seeds gave a yield of 0.035 g/g extractable compounds/dry weight (DW). The ethanol extract was shown to contain 2.91 ± 0.28 mg/g extract or 0.103 ± 0.012 mg/g DW phenolic compounds expressed as gallic acid equivalents (GAE) (Table 1).

Table 1. Total extractable compounds (EC) and total phenolic compounds (PC) (as gallic acid equivalents) in the ethanol extract from the seeds of *A. lividus*

Extract	EC (g /g DW)	PC (mg /g extract)	PC (mg/g DW)	PC/EC (%)
A. lividus seeds ethanol extract	0.035	2.91 ± 0.28	0.103	8.12

Values are the means of three replicates ± standard deviation.

Evaluation of The Antioxidant Activity

The anti-oxidant activity results are presented in Table 2.

Table 2. Antioxidant activities of the ethanol extract from seeds of *A. lividus* species expressed as EC_{50}, TEAC and FRAP values.

Extract	LPO	DPPH·	Superoxide·	ABTS·	TEAC[b]*	FRAP[c]*
	EC_{50} (mg/mL)[a]					
***A. lividus* seeds ethanol extract**	10.06 ± 0.16[a]	48.25 ± 1.630[b]	41.79 ± 0.910[d]	34.39 ± 0.102[c]	2.02 ± 0.04[a]	3.40 ± 0.01[a]
α-Tocopherol	0.112 ± 0.01[b]	0.141 ± 0.012[a]	0.623 ± 0.013[c]	0.428 ± 0.080[d]	2.00 ± 0.05[a]	3.24 ± 0.05[a]
Ascorbic acid	-	0.050 ± 0.004[c]	0.191 ± 0.003[a]	0.146 ± 0.009[b]	2.03 ± 0.01[a]	3.86 ± 0.12[b]
Gallic acid	1.31 ± 0.03[c]	0.032 ± 0.003[d]	0.112 ± 0.016[b]	0.063 ± 0.007[a]	2.02 ± 0.02[a]	3.48 ± 0.32[a]

Values are the means of three replicates ± standard deviation. Values with different letters in the same column were significantly (p <0.05) different.

[a]EC_{50} value: The effective concentration at which the DPPH, ABTS and superoxide radicals were scavenged by 50%.

[b]Expressed as mM Trolox equivalents.

[c]Expressed as mM Ferrous ion equivalents.

*Determined at 80 mg/mL for the extract; 1.25 mg/ml for α-tocopherol; 0.625 mg/mL for ascorbic acid and 0.31 mg/mL for gallic acid

As compared by the EC_{50} values, it was found that the extract was able to scavenge DPPH, $ABTS^{\cdot+}$ and superoxide radicals; however, its antioxidant activity was significantly lower ($p < 0.05$) than that of α-tocopherol, ascorbic acid and gallic acid.

The TEAC value of the extract at a concentration of 80 mg/mL were found to be comparable ($p < 0.05$) to those of α-tocopherol at 1.25 mg/mL, ascorbic acid acid at 0.625 mg/mL, and gallic acid at 0.31 mg/mL. It was found that the extract posesses the reducing power, however it was found to be significantly lower ($p < 0.05$) then that of α-tocopherol, ascorbic acid and gallic acid at the above mentioned concentration.

Evaluation of The Anti-Cholinergic Activity

The ethanol extract was tested for its *in vitro* AChE and BChE inhibitory activities using galantamine as a positive control. The results were expressed as EC_{50} values and are shown in Table 3. It was observed that the extract exhibited AChE and BChE inhibitory activities. However, when compared to the EC_{50} value obtained for the galantamine, the anti-cholinergic activity of the extract was found to be significantly lower ($p < 0.05$).

Evaluation of The Anti-Inflammatory Activity

The inhibitory activity against COX was used to evaluate the anti-inflammatory activity of the extract. The COX-1 and COX-2 inhibitory activities of the extract and indomethacin, used as a positive control, expressed as EC_{50} values are shown in Table 3.

From the EC_{50} values, it was observed that the extract showed inhibitory activities against COX-1 and COX-2. However, the EC_{50} values of the extract were significantly different ($p < 0.05$) from the EC_{50} values obtained for indomethacin (Table 3).

Table 3. AChE, BuChE, COX-1 and COX-2 inhibitory activities of the ethanol extract from the seeds of *A. lividus*.

Extract	EC_{50} (mg/mL)			
	AChE Inhibition	BChE Inhibition	COX-1 Inhibition	COX-2 Inhibition
***A. lividus* seeds ethanol extract**	2.46 ± 2.38[a]	2.54 ± 0.06[a]	4.95 ± 2.12[a]	5.16 ± 1.58[a]
Standard	Galantamine 0.025 ± 0.02[b]	Galantamine 0.039 ± 0.03[b]	Indomethacin 0.0019 ± 0.0002[b]	Indomethacin 0.013 ± 0.003[b]

Values are the means of three replicates ± standard deviation. Values with different letters in the same column were significantly (p <0.05) different.

Purification of Lectin From *A. lividus* Seeds

In this study, we purified the lectin from *A. lividus* seeds in a single chromatography step, which resulted in the appearance of a single peak showing haemagglutinating activity (Figure 1). The lectin was purified approximately 16-fold over the crude extract. The final specific activity of the purified lectin was 1280 HU/mg protein. The results of the purification of lectin from *A. lividus* seeds are summarised in Table 4.

Table 4. Purification of lectin from *A. lividus* seeds (30 g powder).

Purification step	Volume (mL)	Protein (mg/mL)	Total protein (mg)	Haemagglutinating activity (HU)	Specific activity (HU/mg)	Purification fold
Crude extract	245.0	2.03	497.3	39 788.0	80.0	1
50% $(NH_4)_2SO_4$	6.0	1.37	8.22	2630.4	320.0	4
Affinity chromatography	0.9	1.50	1.35	1728.1	1280.0	16.0

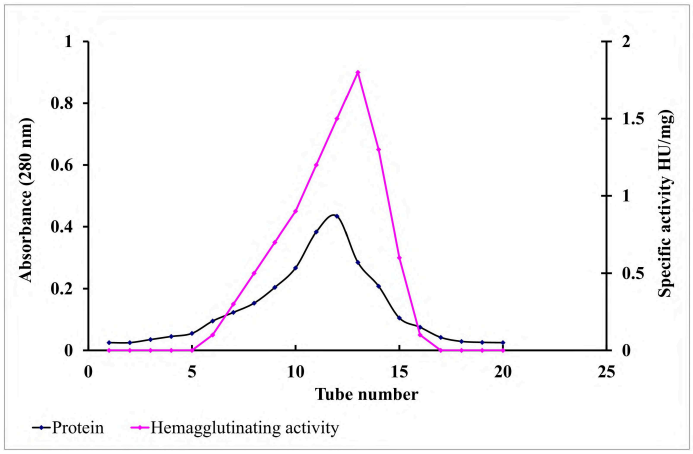

Figure 1. Affinity chromatography elution profile of the active fraction obtained after 50 % ammonium sulphate precipitation of the A. lividus seeds crude extract.

Column: 1 x 14 cm; Sample volume: 6 mL (8.22 mg protein); Elution buffer: 1 M Tris-HCl (pH 7.4) buffer; flow rate: 1 mL/min.

It was observed that the lectin has a complex carbohydrate-binding specificity because its haemagglutinating activity was not inhibited by carbohydrates such as D-lactose, D-mannose, D-fucose, methyl-α-D-mannopyranoside, D-glucosamine hydrochloride, D-raffinose, D-galactose and maltose but, only by glycoprotein (ovalbumin).

SDS-PAGE analysis of purified lectin showed a single band with a native molecular mass of 68 kDa under non-reducing conditions (Figure 2 A) and a single band of 34 kDa under denaturing and reducing conditions (Figure 2 B), revealing that lectin from *A. lividus* seeds is composed of two identical subunits.

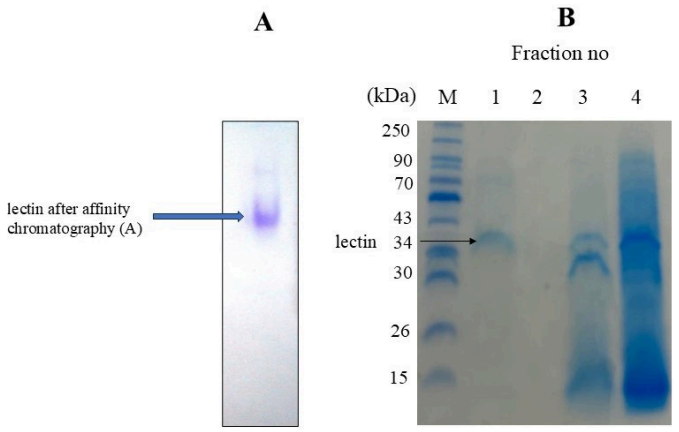

Figure 2. SDS-PAGE of the purified lectin, 50% ammonium sulphate fraction and crude extract

A) Native PAGE showing purified lectin after affinity chromatography

B) SDS-PAGE. M, molecular weight markers; 1, purified lectin after affinity chromatography; 2, empty; 3 – 50 % Ammonium sulphate fraction; 4, crude extract

DISCUSSION

In recent years, the therapeutic value of *Amaranthus* species, which are widely used as food due to their high nutritional value, has been intensively studied. The seeds of *Amaranthus* species are an alternative source of natural fibre, and it was reported that they contain significant amounts of anthocyanins, polyphenols, vitamins and squalene, which may act as antioxidants, thus an Amaranthus-based diet rich in polyphenol compounds may be used as a promising therapeutic agent. Our results were in agreement with the literature and demonstrated that *A. lividus* ethanol seed extract contains phenolic compounds. The amount of total phenolic compounds contained in the *A. lividus* seeds ethanol extract was higher than those determined by Gorinstein et al. (2007) in *A. hypochondriacus*, *A. hybridus* and *A. cruentus* methanol seed extracts, as well as Akin-Idowu et al., (2017) in *A. caudatus*, *A. cruentus*, *A. hybrid*, *A. hypochondriacus* and *A. hybridus* seed extracts; however, the values obtained in this work showed less phenolic content as compared to those reported by Paśko et al. (2009) in *A. cruentus* v. Aztec and *A. cruentus* v. Rawa, as well as Aguilar-Felices et al. (2019) in germinated seed extracts of five *A. cruentus* subspecies.

The extract was tested *in vitro* for its potential to inhibit lipid peroxidation, scavenge radicals, and reduce Fe^{3+} to Fe^{2+}.

The lipid peroxidation inhibitory effect of the *A. lividus* seeds ethanol extract was compared with the effect of α-tocopherol and gallic acid, and it was observed that the extract has an antioxidant effect. These results were in agreement with other studies reporting that Amaranthus species possess lipid peroxidation inhibitory potential (Klimczak et al., 2002).

The superoxide anion radical is like a double-edged sword, on the one hand causing the activation of transcription genes such as NFkB, cell growth and differentiation, and inhibition of apoptosis, on the other hand, it causes oxidative stress leading to apoptosis (Wu et al., 2010). Healthy cells can maintain the balance of the production and inactivation of superoxide radicals. Considering that superoxide radicals can be overproduced by activated macrophages and neutrophils during phagocytic respiratory burst during inflammation via membrane-bound NADPH oxidase enzyme or ischaemia-reperfusion injury (Fujii et al., 2022), in this study, it was concluded that *A. lividus* seeds ethanol extract has superoxide radical scavenging activity and may reduce oxidative damage and balance the free radical production under oxidative stress conditions.

In this study, our findings that *A. lividus* seed ethanol extract converts stable synthetic radicals such as DPPH and ABTS to less reactive species by donating electrons or hydrogen were found to be compatible with the literature (Adegbola et al., 2020).

It has been suggested that iron may play a critical pathophysiological role as a catalyst for free radical formation due to its ability to exist in the stable redox state (Fe^{3+}) and the divalent form (Fe^{2+}), which can transfer an electron and facilitate free radical formation (Markesbery, 1997). Transition metal ions such as Fe^{2+} and Cu^{2+} can induce lipid peroxidation in the cell membrane. In this study, it was concluded that *A. lividus* seeds ethanol extract can reduce Fe^{3+} and thus can donate electrons and participate in lipid peroxidation termination reactions. These findings were found to be consistent with the literature (Adegbola et al., 2020).

Our results indicated a similar antioxidant profile, compared, with stems with leaves and flowers of the same species, which is strongly correlated with their phenolic content (Ozsoy et al., 2009).

The central nervous system (CNS) is high susceptible to free radical damage due to the higher oxygen consumption by the brain, which is enriched with polyunsaturated fatty acids, and the lower activity of antioxidant enzymes compared to other tissues. Alzheimer's disease (AD), which is seen especially in the elderly population and progresses with a decline in cognitive functions, formation of senile amyloid plaques and neurofibrillary tangles, is an irreversible neurodegenerative disease (Anand & Singh, 2013). Numerous studies

have shown that free radicals can mediate neuronal degeneration and death in the AD brain and reported increased oxidative stress biomarkers such as 4-hydroxynonenal and malondialdehyde (MDA), which are the final aldehyde products of lipid peroxidation, protein and DNA oxidation products, and glycosylation end products (AGE) in neurofibrillary tangle and senile plaques.

Cholinesterase inhibitors directly increase cholinergic transmission by inhibiting AChE, an enzyme that breaks down acetylcholine, released from the cholinergic junctions and synapses. In recent studies, it has been determined that BChE acts as a co-regulator of cholinergic neurotransmission. Studies have shown that the activity of BChE is higher in the brains of Alzheimer's patients than in the normal brains, and inhibition of the BChE has gained importance in the treatment. Therefore, AChE and BChE have also been recognised as an important target for treatment of neurodegenerative diseases (Anand & Singh, 2013).

Considering that inhibition of AChE and BChE is the only mechanism by which the success in the symptomatic treatment of AD is achieved, it was concluded that *A. lividus* seeds ethanol extract, which exhibits a strong inhibitory effect on both AChE and BChE activities, can be used as a therapeutic agent against dementia. These results were in agreement with other studies reporting a strong inhibitory effect of polyphenols on AChE activity (Roseiro et al., 2012).

Cyclooxygenases are important enzymes that catalyse the production of prostaglandins (PGs) from arachidonic acid and play a role in inflammation. To investigate the anti-inflammatory and antitumor effects of various compounds, their inhibitory activities on cyclooxygenase enzymes are often measured. While COX-1 is constantly expressed, taking part in physiological processes, upregulated COX-2 expression is frequently increased in many premalignant tissues and malignant tumours. Chronic inflammation is an important risk factor for epithelial carcinogenesis. In areas of chronic inflammation, PGs amounts increase because of cytokine-induced COX-2 induction. This is thought to be one of the reasons why chronic inflammation increases the risk of cancer. Non-steroidal anti-inflammatory drugs (NSAIDs), which are used for the treatment of various inflammatory diseases, inhibit the production of inflammatory mediators from arachidonic acid by blocking COX-1 and COX-2 enzymes (Li et al., 2020).

In this study, the conclusion that *A. lividus* seed ethanol extract has an anti-inflammatory effect by inhibiting COX-1 and COX-2 and can provide an important alternative to classical NSAIDs in the treatment of inflammatory diseases was, consistent with other studies (Caselato-Sousa & Amaya-Farfán, 2017; Jayaprakasam et al., 2004).

A. lividus seeds were also investigated for the presence of lectin. Lectin was purified from the crude extract of *A. lividus* seeds by ammonium sulphate precipitation and affinity chromatography using ovalbumin as a ligand. As lectins are known to be "proteins or glycoproteins of non-immune origin with specific binding affinity for the carbohydrate moiety of glycoconjugates", many lectins have been purified by developing the affinity chromatography method for purification using their ability to bind to dextran-based Sephadex matrices (Tsaneva & Van Damme, 2020). Amaranthin was first isolated and purified to homogeneity from the seeds of *A. caudatus* by a combination of DEAE cellulose ion exchange chromatography and affinity chromatography on T-antigen (Galβ1–3GalNAc) beads (Rinderle & Goldstein, 1989). Different ligands such as stroma from erythrocytes, fetuin, and bovine submaxillary mucin coupled to a matrix (Hernández et al., 1999; Koeppe & Rupnow, 1988; Zenteno & Ochoa, 1988) were reported to be used for the purification of Amaranthus lectins by affinity chromatography.

In this study, the inhibition assay showed that only ovalbumin inhibited the haemagglutinating activity of the *A. lividus* seeds crude extract. With regard to the carbohydrate specificity, *A. lividus* seeds lectin was similar to *A. leucocarpus* lectin, which was inhibited by a glycoprotein fetuin (Hernández et al., 1999; Zenteno & Ochoa, 1988). In this study, it was determined that *A. lividus* seeds lectin is a homodimeric protein exhibiting a native molecular mass of 68 kDa and a subunit molecular mass of 34 kDa, which is within the range from 33-36 kDa of *A. caudatus* (Rinderle & Goldstein, 1989; Koeppe & Rupnow, 1988) and *A. leucocarpus* syn. *hypocondriacus* lectins (Hernández et al., 1999) to 45 kDa per subunit of *A. leucocarpus* lectin (Zenteno & Ochoa, 1988).

CONCLUSION

In this study, it has been shown that *A. lividus* seeds ethanol extract can provide a strong inhibitory effect on the pathological processes caused by oxidative stress due to its reducing as well as hydrogen and electron donating effects, demonstrate AChE and BChE inhibitory activity and thus may have the potential to improve symptoms of Alzheimer's disease, show an anti-inflammatory effect by COX-1 and COX-2 inhibition and thus can provide an important alternative to classical NSAIDs in the treatment of inflammatory diseases. The lectin purified for the first time from *A. lividus* seeds may contribute to the development of lectinological knowledge.

Peer Review Externally peer-reviewed.
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