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Structural Investigation and Antioxidant Activity of Purified Carbohydrate from the Roots of *Amygdalus elaeagnifolia* Spach

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ABSTRACT

Carbohydrates play a crucial role in the food, pharmaceutical, and cosmetic industries, with their applications being closely linked to their specific phytochemical composition. However, a significant knowledge gap exists regarding the phytochemistry of carbohydrates derived from Iran's native and endemic plant species. This study investigates the phytochemical profile and antioxidant activities of purified carbohydrate extracted from the roots of the native Iranian plant, *Amygdalus elaeagnifolia*. were collected from the village of Barshneh, Fars Province, Iran. The roots were first thoroughly cleaned and then subjected to pre-extraction with 96% ethanol. The main carbohydrate extraction was performed using hot water. The extract was subjected to a series of purification steps, including precipitation with 96% ethanol, protein removal using Sevag method, ion-exchange chromatography with DEAE cellulose as the stationary phase, and gel filtration chromatography using Sephadex G-100. Following these steps the purified carbohydrate, designated as AEC-1, was successfully obtained. The functional group of AEC-1 were analyzed using Fourier-transform infrared (FTIR) spectroscopy. The monosaccharide composition was determined using gas chromatography-flame ionization detection (GC-FID), while monomer identification was conducted using gas chromatography-mass spectrometry (GC-MS). Finally, the antioxidant capacity of AEC-1 was evaluated against three free radicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and hydroxyl radical. The purity of AEC-1 was determined to be 94.08 + 0.67%. FT-IR analysis demonstrated functional groups such as an acid group, α-anomeric carbons and the pyranose ring structures. Chromatographic studies demonstrated the following monosaccharide profile: arabinose (31.52%), galactose (26.10%), mannose (19.00%), glucose (16.17%), fructose (4.63%), and glucuronic acid (2.56%). Antioxidant assays demonstrated significant, concentration-dependent inhibitory effect of AEC-1 on DPPH, ABTS, and hydroxyl radicals. The results indicate that the purified carbohydrate from *A. elaeagnifolia* species is an acidic heteropolysaccharide with moderate antioxidant activity against DPPH, ABTS, and hydroxyl radicals.

1.Introduction

Plant-derived carbohydrates represent an important group of compounds, which have gained great interest over the last few years. The interest is due to their multiple applications in the pharmaceutical, food, and cosmetic industries, coupled with their many biological activities. Some of these beneficial effects include activities that are anti-cancer, anti-aging, anti-obesity, antioxidant, anti-inflammatory, and immunostimulant. Structurally, these compounds are a mixture of many monosaccharides, oligosaccharides, and polysaccharides, which can be readily isolated from plant organs [1-3].

Polysaccharides and other carbohydrate molecules in plants can exhibit high solubility in water because of their polarity. Different techniques are used for extracting these substances, including microwave extraction, ultrasonic extraction, acid/alkali extraction, and enzymatic extraction. In addition to these, hot water extraction is regarded as a fundamental and common technique in the field [2-3]. The classical hot water extraction and alcohol precipitation technique has been the technique of choice for researchers due to its simplicity and astounding efficiency [4]. Here, macromolecules such as polysaccharides that are insoluble in alcohol precipitate out and may then be physically separated. Purification of the carbohydrates is done by several methods, such as pre-extraction with organic solvents, elimination of proteins and nucleic acids by the Sevag method, ethanol precipitation, and application of anionic resins and Sephadex gels [5]. After purification, the compounds are identified and determined by spectroscopic and chromatographic methods [6].

Amygdalus is a genus of the Rosaceae family, comprising thirty species that are extensively dispersed throughout Central Asia that contain tree and shrub morphologies. There are twenty-one *Amygdalus* species in Iran. These species hold considerable ecological and economic significance, largely attributable to their inherent resistance to drought conditions, which renders them invaluable components of natural landscapes and mountainous ecosystems [7]. Traditionally in herbal medicine, *Amygdalus* species have been utilized in the alleviation of muscular, skeletal, joint pain, the removal of chronic gastrointestinal mucosal secretions, and the mitigation of headaches attributed to a cold temperament [8, 9].

Previous studies have revealed various pharmacological activities of *Amygdalus* species. The ethanolic extract from *Prunus amygdalus* var. amara has demonstrated notable anti-cancer activity against a number of human cancer cell lines, such as U937, T-24, PC-3, SKOV-3, A-549, Huh-7, NB4, APL, and Hep-2 [10]. Furthermore, a purified carbohydrate from *Prunus amygdalus* gum, composed of galactose, arabinose, xylose, mannose, rhamnose, and glucuronic acid in the molar ratios of 45, 26, 7, 10, 1, and 11, respectively, has exhibited antibacterial activity against several significant bacterial pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. This carbohydrate also showed antioxidant activity with an IC_{50} value of 6.6 mg/mL in the DPPH assay [11]. Carbohydrates isolated from *Amygdalus persica* L., composed of rhamnose, arabinose, mannose, and glucose with a relative percentage of 0.17, 0.034, 1, and 0.17, respectively, demonstrated significant scavenging activity against DPPH (85.45 ± 0.12% at 400 µg/ml), ABTS (99.96 ± 0.066% at 60 µg/ml), and superoxide radicals (18.13 ± 0.59% at 80 µg/ml). The antioxidant activity of this carbohydrate against hydroxyl (OH) radicals increased from 64.15 ± 0.42% at 10 µg/ml to 89.74 ± 0.98% at 50 µg/ml. Notably, within the concentration range of 10 to 125 µg/ml, free radical scavenging activity was better than that of vitamin C [2]. Another study reported the monosaccharide composition

of a purified carbohydrate from *Amygdalus scoparia* Spach gum, which contains arabinose, rhamnose, mannose, fructose, galactose, glucuronic acid, galacturonic acid, and glucose in a relative percentage of 25.32, 9.01, 11.54, 10.82, 22.04, 3.85, 5.88, and 5.16, respectively [12]. Moreover, a carbohydrate fraction purified from *Prunus amygdalus* composed of galactose, arabinose, mannose, and xylose with molar ratios of 61.4, 32.2, 2.9, and 2.68 respectively, demonstrated the ability to inhibit DPPH and ABTS radicals, with inhibition percentages of 61.58 ± 2.3% and 44 ± 1.1% at 1.25 mg/ml, respectively [13].
Amygdalus elaeagnifolia, commonly known as Arjan or Kerman almond, is a native Iranian plant species that grows in different provinces, including Kerman, Fars, Kohgiluyeh and Boyer-Ahmad, Chaharmahal and Bakhtiari, Lorestan, and Arak. The plant is normally a shrub or small tree and has been used conventionally in traditional medicine to alleviate dermatological complications of radiotherapy as well as to assist in reducing stress [7,14].
There is limited phytochemical information available for *A. elaeagnifolia*. Iranmanesh et al. (2017) reported a study of the essential oil of its fruit, identifying several fatty acids including myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, gadoleic, and behenic acids [15]. Studies have shown there are no reports on *A. elaeagnifolia* carbohydrates and their associated bioactivities. Due to the importance of phytochemical studies on native and endemic plants in Iran, the objective of the present study was to extract, purify, and characterize a carbohydrate from *A. elaeagnifolia*. The extraction was carried out using hot water, followed by purification using different procedures such as solvent pre-extraction, the Sevag method, and ion-exchange chromatography. The purified carbohydrate was identified using Fourier-transform infrared spectroscopy (FT-IR), gas chromatography coupled with flame ionization detection (GC-FID), and gas chromatography-mass spectrometry (GC-MS). Additionally, the antioxidant activity of the isolated carbohydrate was evaluated against DPPH, ABTS, and hydroxyl (OH) radicals.

2- Materials and methods

2.1. Plant material

Amygdalus elaeagnifolia Spach roots were collected and identified in June 2019 by Dr. Mojtaba Asadollahi (botanist) from the vicinity of Barshaneh village, a part of Sepidan County, Fars Province, Iran (N 30° 17’, E 51° 55’), 2478 meters above sea level. A voucher specimen with the number MPH-3219 was placed to the Herbarium of the Medicinal Plants and Drugs Research Institute at Shahid Beheshti University, Tehran, Iran.

2.2. Material and chemical

Sephadex G-100 and DEAE-Cellulose A52 were purchased from Pharmacia Co. (Uppsala, Sweden). D-galactose, L-arabinose, D-fructose, D-glucose, D-mannose, D-xylose, and L-rhamnose were provided from Bio Basic Canada Inc. (Markham, Canada). Methoxyamine hydrochloride, DPPH and ABTS were obtained from Sigma-Aldrich Chemical Co., Ltd. (Louis, USA). *N*, *O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane, trifluoroacetic acid (TFA), hydrogen peroxide, salicylic acid, iron(II) sulfate, pyridine and potassium persulfate, were purchased from Merck Company (Darmstadt, Germany). hydrochloric acid (37%), n-butanol and chloroform were obtained from Carlo Erba Company (Val-de-Reuil, France). This test used analytical grade chemicals.

2.3. Extraction of *A. elaeagnifolia* carbohydrate

The extraction of A. elaeagnifolia root involved an initial reflux with 1000 mL of 96% (v/v) ethanol for 1 hour, a process repeated thrice with supernatant removal. Following this, the remaining residue underwent three successive extractions, each using 1000 mL of distilled water at temperature of 70°C for 1 hour. All collected supernatants were combined, filtered, and then concentrated utilizing rotary evaporator (Heidolph Laborota 4000 efficient, Germany). The aqueous extract was subsequently precipitated by adding four volumes of 96% ethanol at 4°C for 24 hours. The resulting precipitate was isolated through centrifugation at 4000 rpm for 20 minutes. Deproteinization was achieved by three repetitions of the Sevag method (n-butanol/chloroform, 1:4 v/v). Finally, the supernatant was freeze-dried, yielding 2.1 g of crude *A. elaeagnifolia* carbohydrate (AEC). For purification, 2 g of AEC was dissolved in deionized water, passed through a membrane filter (0.45 µm), and subsequently loaded onto a DEAE-52 cellulose column (2.6 × 30 cm). The column was then eluted in a stepwise manner with 0, 0.1, 0.15, 0.20, 0.25, and 0.3 M NaCl solutions at a consistent flow rate of 2.5 mL/min. Each fraction (4 mL) was monitored for the presence of carbohydrates utilizing the phenol sulfuric acid method at 490 nm. The predominant carbohydrate fractions were further subjected to a Sephadex G-100 column (1.6 cm × 70 cm) and eluted with distilled water. The major peak was combined, freeze-dried, and designated as an isolated carbohydrate (65 mg), named AEC-1, which was then used for subsequent investigations [16].

2.4. UV–vis and FT-IR analysis

A UV-Vis spectrophotometer (Shimadzu UV-2501PC, Japan) quantified nucleic acids at 260 nm and proteins at 280 nm, individually [16]. Organic functional groups were identified by an FT-IR spectrometer (Tensor 27, Bruker, USA) in the range of 4000–400 cm^{-1} [17].

2.5. Carbohydrate content and monosaccharide composition

The non-reducing sugar content was determined by calculating the difference between the total and reducing sugar content in AEC-1 [18]. Total sugar quantification was performed using the phenol-sulfuric acid method, with D-glucose serving as the reference standard. Reducing sugar content was measured by the DNS method at a wavelength of 575 nm [19]. The purity of AEC-1 was assessed using the following formula:

Purity = (non-reducing carbohydrate content/dry mass of AEC-1) ×100 (1)

To analyze the monosaccharide composition, 5 mg of AEC-1 was hydrolyzed using 2 M TFA at 120°C for 90 minutes. The resulting hydrolysate underwent oxime formation by reaction with 50 µL of methoxyamine hydrochloride (20 mg/mL in pyridine) at 80°C for 20 minutes. Subsequently, derivatization was completed by adding 49.5 µL of BSTFA and 1 µL of trimethylchlorosilane, followed by heating at 80°C for 20 minutes. Derivatized samples then underwent GC-FID analysis using a Thermoquest-Finnigan instrument (Thermo Fisher Scientific, USA) fitted with a 60 m × 0.25 mm ID × 0.25 µm DB-5 fused silica column. The GC oven temperature began at 60°C for 5 minutes, then ramped to 280°C at 5°C/min, holding for 11 min. Nitrogen served as the carrier gas at 1.1 mL/min. Injector and detector temperatures were set to 250°C and 280°C, respectively. Complementary GC–MS analysis was conducted on a Thermoquest-Finnigan Trace GC–MS device (Thermo Fisher Scientific, USA), employing the same chromatographic parameters. Helium was used as the carrier gas for GC-MS at 1.1 mL/min. Monosaccharide identification was accomplished by comparing the mass spectra and retention indices of AEC-1 derivatives against those of authentic TMSi monosaccharide standards analyzed under identical GC conditions. Further validation was achieved by cross-referencing mass spectra with published literature data and the Golm Metabolome Database (GMD) [18].

2.6. Antioxidant activities analysis

2.6.1. DPPH radical scavenging activity

The antioxidant capacity of AEC-1 was assessed using a modified Blois method [26]. Briefly, AEC-1 was prepared in distilled water across a concentration range of 0 to 10 mg/mL. Subsequently, 50 µL of each AEC-1 solution was combined with freshly DPPH solution (200 µL). Following brief agitation, the resulting sample solutions were held at ambient temperature in darkness for 30 minutes. The absorbance (A) of these solutions was then recorded at 517 nm, with ascorbic acid serving as a positive control. The percentage of DPPH radical scavenging activity was calculated as follows [27]:

Scavenging activity% = ((Ac– As)/Ac) ×100 (2)

Ac represents the absorbance of the solution devoid of the samples, whereas AS denotes the absorbance of the sample solutions at varying concentrations.

2.6.2. Assay of ABTS radical scavenging ability

The ABTS radical scavenging capacity of AEC-1 was determined by adapting a previously published protocol with minor adjustments [28, 29]. The ABTS radical solution was formulated by blending ABTS (7.0 mM) and potassium persulfate (2.45 mM) in equal volumes. This mixture was then incubated at 25°C in complete darkness for 24 hours. The stock solution was diluted with phosphate buffer (10 mM, pH 7.4) until its absorbance at 734 nm reached a stable value of 0.700 ± 0.020. For the assay, 10 µL of the AEC-1 sample was introduced into 290 µL of the diluted ABTS working solution. After a 10-minute incubation period at 25°C, the absorbance of the reaction mixture was measured spectrophotometrically at 734 nm. Vitamin C was included as a positive control. The ABTS radical scavenging activity was computed using the identical formula employed for the DPPH radical scavenging assay.

2.6.3. Hydroxyl radical scavenging activity

The scavenging activity against hydroxyl radicals was assessed in accordance with a previously established methodological approach [30]. A reaction medium was constituted by combining 1 mL of AEC-1 solution (at designated concentrations of 1 to 10 mg/mL), 1 mL of 6 mM ethanol salicylic acid and 1 mL of 6 mM FeSO4. This mixture was thoroughly agitated within a test tube and subsequently allowed to equilibrate for 10 minutes. Following this, 1 mL of 2.4 mM hydrogen peroxide was introduced, and the entire mixture was incubated for 30 minutes at 37°C in a without light environment. Sample absorbance was then measured at 510 nm. Vitamin C (VC) was used as a positive reference. The percentage of hydroxyl radical scavenging activity (RSA%) was quantified utilizing the subsequent formula:

Hydroxyl RSA% = 1 - $(A_2$ - $A_1)/A_0$ ×100

(3) Where A2 is sample absorbance, A1 is absorbance without ethanol-salicylic acid, and A0 is absorbance without any samples

2.7. Statistical analysis

All experimental data are presented as the mean ± standard deviation (SD). Statistical analyses were performed through one-way ANOVA utilizing Microsoft Excel 2016. A P-value below 0.05 was considered indicative of statistical significance.

3-Results

3.1. Isolation and purification of AEC-1

The initial crude carbohydrate obtained from the roots of *A. elaeagnifolia* was extracted using warm water, subsequently precipitated with organic solvent, and subjected to deproteinization through the Sevag method. This preliminary extraction yielded a crude carbohydrate with a yield of 2.1%. Subsequent purification steps involved on ion exchange column and Sephadex G-100 gel. Fractions 18 to 36, which constituted the peak observed during elution from the DEAE-cellulose A52 column (Fig. 1A), were combined and subsequently loaded on Sephadex G-100. Fractions 48_65 collected from Sephadex G-100 (Fig. 1B) were designated as AEC-1 for further analysis. Ultimately, the yield of AEC-1 was about 3%.

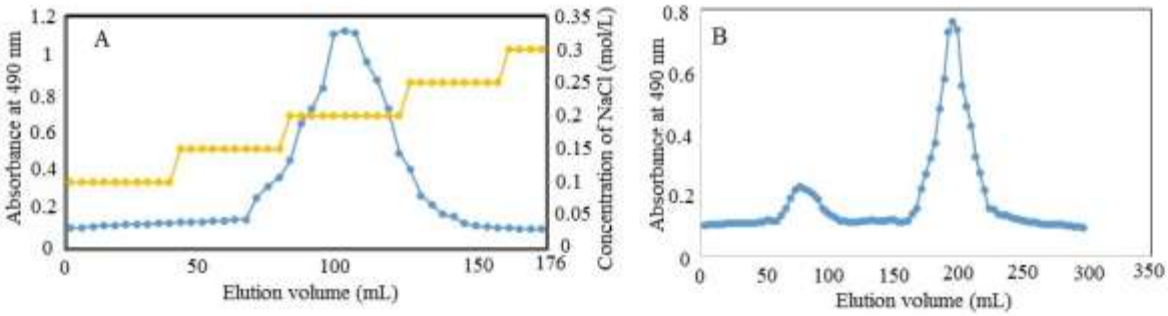

Fig 1. (A) Elution profile illustrating the fractions collected from the DEAE-A52-cellulose column (B) Elution profile of the collected fractions from Sephadex G-100

3.2. UV and FT-IR spectrum analysis

The UV-Vis spectrum did not exhibit any absorption peaks at wavelengths of 280 and 260 nm, thereby suggesting the non-existence of nucleic acids and proteins in AEC-1 (Fig. 2B) [27].

Analysis of the FT-IR spectrum of AEC-1 (Fig. 2A) revealed several distinctive absorption bands. Peaks at 3399 cm^{-1}, 2927 cm^{-1}, and around 1600 cm^{-1} (1626, 1669), correspond to the OH groups stretching, the C–H groups stretching, and the COO bond vibration, respectively [16]. Furthermore, vibrations of C-H groups were identified by bands at 1362 cm^{-1} and 1455 cm^{-1}. The presence of alcoholic C-O groups was supported by absorption bands at 1046 cm^{-1} and 1082 cm^{-1}. The absorptions at 833 and 781 cm^{-1} were ascribed to α-configurations of carbohydrate units and pyranosyl residues [28].

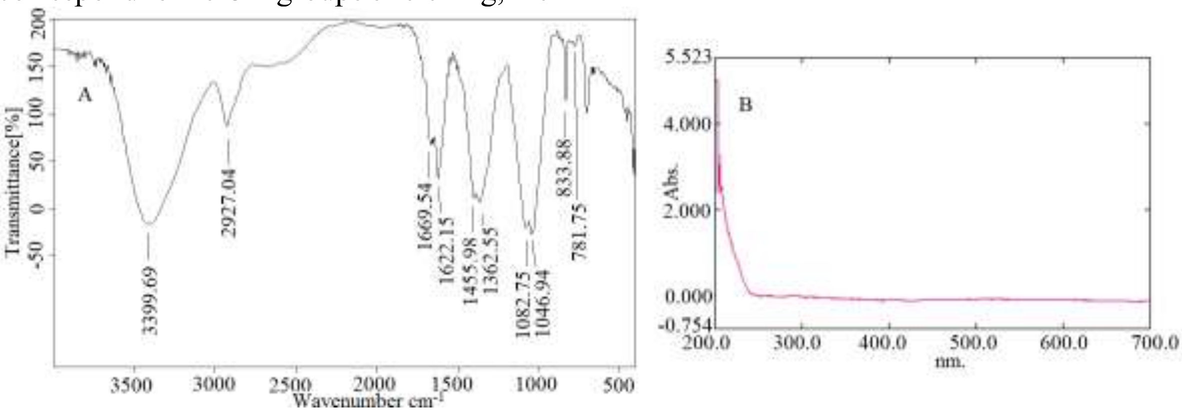

Fig 2. (A) FT-IR spectrum of AEC -1 (B) UV-Vis spectrum of AEC -1

3.3. Determination of carbohydrate content and monosaccharide composition of AEC-1

The total carbohydrate content of AEC-1 was 94.08±0.67, determined using the phenol-sulfuric acid method. Conversely, the DNS method indicated the absence of reducing sugars. Component Monosaccharide composition analysis was conducted following acid hydrolysis and derivatization, with subsequent detection and quantification performed by GC-FID and GC-MS analyses (Fig. 3A and B). AEC-1 was identified as a heteropolysaccharide. Its constituent monosaccharides and their relative percentages were: Arabinose (Ara) at 31.52%, Galactose (Gal) at 26.10%, Mannose (Man) at 19.00%, Glucose (Glc) at 16.17%, Fructose (Fruc) at 4.63%, and Glucuronic Acid (GlcA) at 2.54%. The GlcA content in the monosaccharide composition was approximately 2.5%, confirming that AEC-1 was an acidic carbohydrate. The GC chromatogram may display two distinct peaks for each monomer due to the methoximation of carbonyl groups, which generates both syn- and anti-products [18].

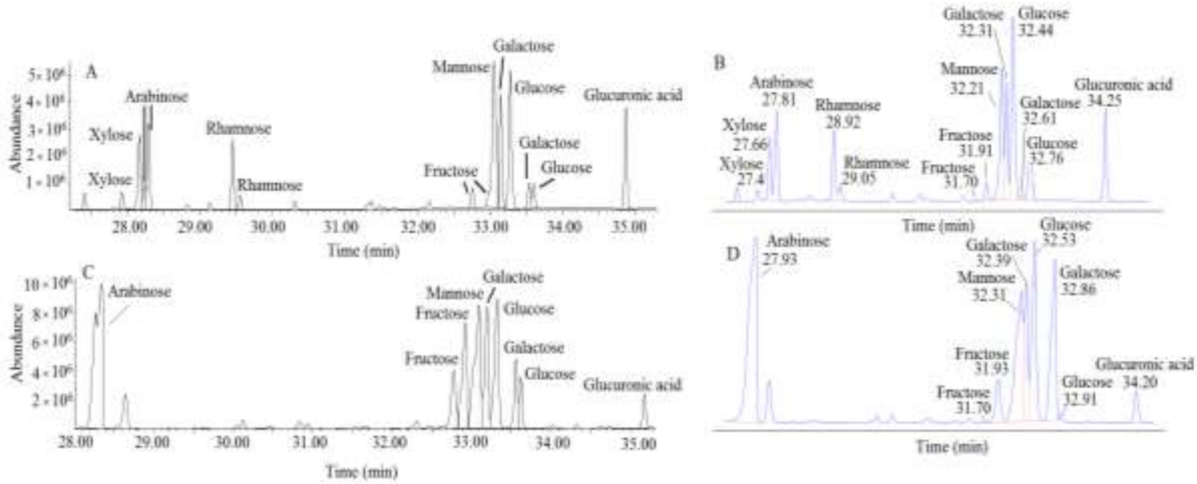

Fig 3. GC chromatograms. (A) GC–MS chromatogram of standard monosaccharides, (B) GC-FID chromatogram of standard monosaccharides, (C) GC–MS chromatogram of AEC-1 (D) GC-FID chromatogram of AEC-1

3.4. Antioxidant activity of AEC-1

3.4.1. DPPH radical scavenging activity

The DPPH scavenging capability of AEC-1 was assessed at a wavelength of 517 nm (Fig. 4A) [29]. At a concentration of 3.5 mg/mL, AEC-1 exhibited a scavenging activity of 39.48%. The IC_{50} for AEC-1 was determined to be 5.59 ± 0.58 mg/mL.

3.4.2. Assay of ABTS radical scavenging ability

The ABTS assay serves as a methodology for evaluating the comprehensive antioxidant capacity of both isolated compounds and intricate mixtures [30]. Figure 4B illustrated the ABTS free radical scavenging effect of AEC-1 increased in a concentration-dependent manner. At a concentration of 3.5 mg/mL, AEC-1 demonstrated a scavenging activity of 34.05%. Its IC_{50} value was established at 6.47 ± 0.62 mg/mL.

3.4.3. Hydroxyl radical scavenging activity

OH radicals are potent oxidizing free radicals known to inflict damage upon various biomolecules, including proteins, carbohydrates, nucleic acids, and lipids, ultimately culminating in cellular injury and tissue destruction [26, 30]. The outcomes regarding the antioxidant activity (OH) of AEC-1 were presented in Fig. 4C. The scavenging effect of AEC-1 was unequivocally demonstrated to be concentration -dependent. At a concentration of 3.5 mg/mL, the scavenging activity of AEC-1 was 44.13%, and IC_{50} value was 4.84 ± 0.62 mg/mL.

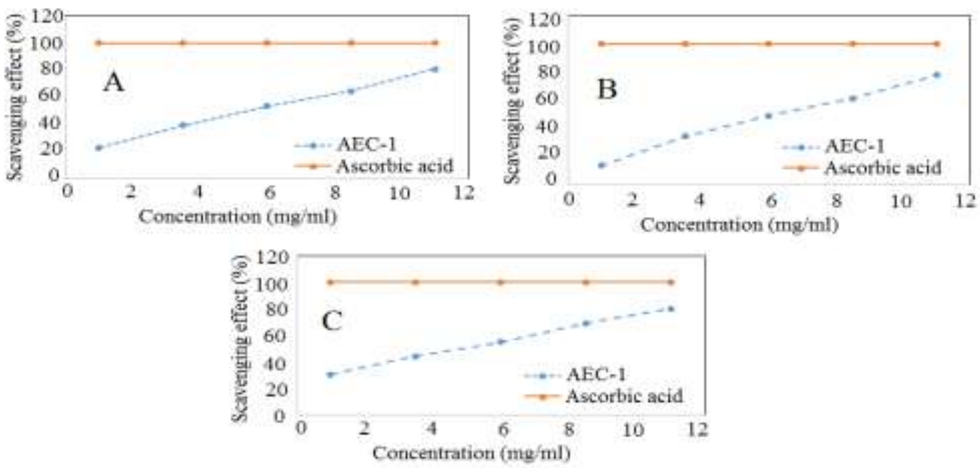

Fig 4. Scavenging effects AEC-1: (A) DPPH radical scavenging activity, (B) ABTS radical scavenging ability, (C) hydroxyl radical scavenging activity. Data are expressed as mean ± standard deviation (n=3)

4- Discussion

The genus *Amygdalus* includes a series of species; however, only a limited number have been thoroughly investigated for their carbohydrate phytochemistry. The findings presented in this study indicate that AEC-1 is a heteropolysaccharide, with galactose and arabinose constituting the most abundant monosaccharide components. These observations aligned with previous reports for *Amygdalus scoparia* Spach gum, *Prunus amygdalus* gum and *Prunus amygdalus* peels [11-13, 31]. In the AEC-1 sample, the arabinose monosaccharide percentage (31.52%) is higher than that of galactose (26.10%). This finding is consistent with the neutral carbohydrate purified from *Amygdalus scoparia* Spach gum, which contains 20% arabinose and 17.9% galactose. Similarly, the acidic carbohydrate purified from *Amygdalus scoparia* Spach gum also shows a higher arabinose content (25.32%) compared to galactose (22.04%). In contrast, the gum and peels of *Prunus amygdalus* exhibit a higher galactose content (45% and 61.4%, respectively) than arabinose (26% and 32.2%, respectively) [11-13, 31]. Furthermore, the monosaccharides of AEC-1 are similar to those of purified monosaccharides from *Amygdalus scoparia* Spach gum, although significant differences were observed in their molar ratios; rhamnose and galacturonic acid were not detected in the root of *Amygdalus elaeagnifolia* [12].

Investigation into the antioxidant activities of AEC-1 revealed a direct correlation between increasing carbohydrate concentration and enhanced scavenging efficacy against DPPH, ABTS, and OH radicals (dose-dependent manner). AEC-1 exhibited higher antioxidant activity in scavenging DPPH radicals (IC_{50} of 5.59 ± 0.58 mg/mL), compared to the purified carbohydrate from *Prunus amygdalus* gum (IC_{50} of 6.6 mg/mL) [11]. On the other hand, AEC-1 showed inferior antioxidant activity in scavenging DPPH and ABTS radicals (IC_{50} of 5.59 ± 0.58 mg/mL and 6.47 ± 0.62 mg/mL, respectively) relative to purified carbohydrate from *Prunus amygdalus* kernel, DPPH and ABTS radical inhibition at 1.25 mg/mL (61.58 ± 2.3% inhibition) and 1.25 mg/mL (44 ± 1.1% inhibition), respectively [13]. Similarly, AEC-1 showed less potent inhibition of DPPH, ABTS, and OH radicals compared to carbohydrates isolated from *Amygdalus persica* L., which displayed antioxidant activities with IC_{50} values of 400 µg/mL (85.45 ± 0.12% inhibition), 60 µg/mL (99.96 ± 0.066% inhibition), and 10 µg/mL (64.15 ± 0.42% inhibition), respectively [2]. The antioxidant activity of carbohydrates is influenced by several factors, including monosaccharide composition, molecular weight, the quantity and position of uronic acids, conformation, and overall structural [31]. Arabinose, mannose, and glucose are considered key units contributing to antioxidant activity [32, 33].

5- Conclusion

Amygdalus elaeagnifolia is a native Iranian plant species, and there are no reports on *A. elaeagnifolia* carbohydrates and their associated bioactivities. In this study, AEC-1 was isolated and purified from *A. elaeagnifolia* root. GC-MS and GC-FID determined this carbohydrate has six monosaccharides which arabinose and galactose account for more than 50% of the carbohydrate. Additionally, other monosaccharides such as mannose, glucose, fructose, and glucuronic acid are present in this carbohydrate. The relative proportion of the monosaccharides by GC-FID analysis was 52.31% arabinose, 10.26% galactose, 19.00% mannose, 16.17% glucose, 4.63% fructose, and 2.56% glucuronic acid, respectively.

The presence of glucuronic acid as a monomer indicates that AEC-1 is an acidic carbohydrate. Furthermore, *in vitro* evaluations of its DPPH, ABTS, and OH radical scavenging activities demonstrated that AEC-1 possesses moderately acceptable antioxidant capabilities. To gain a more comprehensive understanding of the medicinal effects attributed to this species, further investigations are warranted. These include: (1) comprehensive elucidation of the carbohydrate's backbone and branching structures; (2) precise determination of the linkages present between monosaccharide units within AEC-1; and (3) detailed characterization of the biological activity and underlying antioxidant mechanisms of the purified carbohydrate.

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Declaration competing interest
The authors have no conflicts of interest to declare.
Availability of data and material
Data is available.
Code availability
Not application.
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6-References

[1] Lu, X., Jing, Y., Zhang, N., Chen, L., Tai, J. and Cao, Y., 2025. Structural characterization and anti-obesity effect of a novel water-soluble galactomannan isolated from *Eurotium cristatum*. Carbohydrate Polymers, 348(Part B): 122870.

[2] Yu, Q., Li, W., Liang, M., Li, G., Wu, Z., Long, J., Yuan, C., Mei, W. and Xia, X., 2024. Preparation, characterization, and antioxidant activities of extracts from *Amygdalus persica* L. Flowers. Molecules, 29(3): 633.

[3] Ji, C., Ma, Y., Xie, Y., Guo, J., Ba, H., Zhou, Z., Zhao, K., Yang, M., He, X. and Zheng, W., 2024. Isolation and purification of carbohydrate components in functional food: a review. RSC Advances, 14(32): 23204–23214.

[4] Wang, X., Lu, Y., Li, M., Xia, X., Jin, C., Ding, K. and Chen, D., 2024. Structural characterization and Bacteroides proliferation promotion activity of a novel homogeneous arabinoglucuronoxylan from *Commelina communis* L. Bioorganic Chemistry, 153: 107790.

[5] Hao, J., Zhu, Y., Zhang, Y., Li, L., Li, Z., Wang, L., Qu, Y., Qi, L., Yu, H. and Wang, D., 2025. Structural characterization and hypolipidemic activity of a hetero-galactan purified from *Sanghuangporus vaninii* based on modulation of TLR4/NF-κB pathway. Carbohydrate Polymers, 347(1): 122702.

[6] Cao, L., Liu, W., Jing, H., Yasen, A., Wang, J., Wang, Y., Yang, Z., Yili, A., Nuerxiati, R. and Weng, Z., 2024. Ultrasound-assisted low-temperature extraction of polysaccharides from *Lavandula angustifolia* Mill.: optimization, structure characterization, and anti-inflammatory activity. International Journal of Biological Macromolecules, 282(2): 136764.

[7] Saberi, F., Kiani, B., Omidvar, E., Azimzadeh, H. and Esmaeilpour, M., 2023. Evaluating the plantation success by mountain almond (*Amygdalus scoparia* Spach.) and its effect on vegetation and soil in Arjan habitats of Jamal Beyg region, Fars province. Water and Soil Management and Modeling, 3(4): 227–241.

[8] Kianbakht, S., Nabati, F. and Hashem Dabaghian, F., 2023. Effects of topical Persian medicine *Amygdalus communis* L. var. Amara kernel oil on the symptoms of knee osteoarthritis: a randomized, triple-blind, active, and placebo-controlled clinical trial. Journal of Medicinal Plants, 22(87): 89–96.

[9] Sharifpoor, R., Jafari, A. and Jhanbazi Gojani, H. 2016. Effects of aspect on age dependent quality and quantity of mountain almond (*Amygdalus arabica* Olivier) oil (case study: Karebas, Cheharmahal-va-Bakhtiary). Iranian Journal of Medicinal and Aromatic Plants, 32(4): 688–697.

[10] Shalayel, M. H. F., Al-Mazaideh, G. M., Alanezi, A. A., Almuqati, A. F. and Alotaibi, M., 2023. The potential anti-cancerous activity of *Prunus amygdalus* var. amara extract. Processes, 11(4): 1277.

[11] Bouaziz, F., Koubaa, M., Helbert, C. B., Kallel, F., Driss, D., Kacem, I., Ghorbel, R. and Chaabouni, S. E., 2015. Purification, structural data and biological properties of polysaccharide from *Prunus amygdalus* gum. International Journal of Food Science &

Technology, 50(3): 578–584.

[12] Seyfi, R., Kasaai, M. R. and Chaichi, M. J., 2019. Isolation and structural characterization of a polysaccharide derived from a local gum: Zedo (*Amygdalus scoparia* Spach). Food Hydrocolloids, 87: 915–924.

[13] Dammak, M. I., Chakroun, I., Mzoughi, Z., Amamou, S., Mansour, H. Ben, Le Cerf, D. and Majdoub, H., 2018. Characterization of polysaccharides from *Prunus amygdalus* peels: Antioxidant and antiproliferative activities. International Journal of Biological Macromolecules, 119: 198–206.

[14] Hassanpouraghdam, M. B., Ghorbani, H., Esmaeilpour, M., Alford, M. H., Strzemski, M. and Dresler, S., 2022. Diversity and distribution patterns of endemic medicinal and aromatic plants of Iran: Implications for conservation and habitat management. International Journal of Environmental Research and Public Health, 19(3): 1552.

[15] Iranmanesh, Y. and Jahanbazi Goujani, H., 2017. Comparison of fatty acid composition of fruit oil from acorn (Persian oak), Pistacia atlantica Desf. and four wild almond species. Iranian Journal of Medicinal and Aromatic Plants, 33(2):325–337.

[16] Cai, L., Zou, S., Liang, D. and Luan, L., 2018. Structural characterization, antioxidant and hepatoprotective activities of polysaccharides from *Sophorae tonkinensis* Radix. Carbohydrate Polymers, 184(15): 354–365.

[17] Zheng, X., Liu, Z., Li, S., Wang, L., Lv, J., Li, J., Ma, X., Fan, L. and Qian, F., 2016. Identification and characterization of a cytotoxic polysaccharide from the flower of *Abelmoschus manihot*. International Journal of Biological Macromolecules, 82: 284–290

[18] Ahmadi, E., Rezadoost, H., Alilou, M., Stuppner, H. and Moridi Farimani, M., 2022. Purification, structural characterization and antioxidant activity of a new arabinogalactan from *Dorema ammoniacum* gum. International Journal of Biological Macromolecules, 194: 1019–1028.

[19] Miller, G. L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3): 426–428.

[20] Blois, M. S., 1958. Antioxidant determinations by the use of a stable free radical. nature, 181(4617): 1199–1200.

[21] Asghari, B., Mafakheri, S. and Zarabi, M., 2019. Study of antioxidant and antimicrobial activity of date seed extract and its effects on physicochemical, microbial and sensory properties of cupcake. Journal of food science and technology(Iran), 16(88): 327–342.

[22] Ghorbani, Sh., Jafarian, S., Sharifi Soltani, M. and Roozbeh nasiraie, L., 2025. Evaluation of some physicochemical and sensory properties of beef sausage containing sour tea (*Hibiscus Sabdariffa* L .) Extract. Journal of food science and technology(Iran), 22(58): 63-79.

[23] Yang, C., Hu, C., Zhang, H., Chen, W., Deng, Q., Tang, H. and Huang, F., 2020. Optimation for preparation of oligosaccharides from flaxseed gum and evaluation of antioxidant and antitumor activities in vitro. International Journal of Biological Macromolecules, 153:1107–1116.

[24] Jing, C., Yuan, Y., Tang, Q., Zou, P., Li, Y. and Zhang, C., 2017. Extraction optimization, preliminary characterization and antioxidant activities of polysaccharides from *Glycine soja*. International Journal of Biological Macromolecules, 103: 1207–1216.

[25] Salmanian, Sh., Sadeghi Mahoonak, A.R. and Jamson, M., 2018. Determination of amounts, antioxidant properties and identification of main phenolic compound in Enarijeh (*Froriepiasubpinnata*) extract by RP-HPLC method. Journal of food science and technology(Iran), 15(81): 287-297.

[26] Liu, Q., Ge, X., Chen, L., Cheng, D., Yun, Z., Xu, W. and Shao, R., 2018. Purification and analysis of the composition and antioxidant activity of polysaccharides from *Helicteres angustifolia* L. International Journal of Biological Macromolecules, 107(Part B): 2262–2268.

[27] Jahanbin, K., 2018. Structural characterization of a new water-soluble polysaccharide isolated from *Acanthophyllum acerosum* roots and its antioxidant activity. International Journal of Biological Macromolecules, 107(Part A): 1227–1234.

[28] Mathlouthi, M. and Koenig, J. L., 1987. Vibrational spectra of carbohydrates. advances in carbohydrate chemistry and biochemistry, 44: 7-89

[29] Hou, Y., Ding, X. and Hou, W., 2015. Composition and antioxidant activity of water-soluble oligosaccharides from *Hericium erinaceus*. Molecular Medicine Reports, 11(5):

3794–3799.
[30] Fan, H., Meng, Q., Xiao, T. and Zhang, L., 2018. Partial characterization and antioxidant activities of polysaccharides sequentially extracted from *Dendrobium officinale*. Journal of Food Measurement and Characterization, 12(2): 1054–1064.
[31] Molaei, H. and Jahanbin, K., 2018. Structural features of a new water-soluble polysaccharide from the gum exudates of *Amygdalus scoparia* Spach (Zedo gum). Carbohydrate Polymers, 182: 98–108.
[32] Zeng, C., Chen, X., Jiang, W., Liu, L. and Fanga, C., 2020. Isolation, purification and antioxidant activity of the polysaccharides from chinese truffle *Tuber sinense*. Iranian Journal of Pharmaceutical Research, 19(1): 436–447.
[33] Lo, T. C.-T., Chang, C. A., Chiu, K.-H., Tsay, P.-K. and Jen, J.-F., 2011. Correlation evaluation of antioxidant properties on the monosaccharide components and glycosyl linkages of polysaccharide with different measuring methods. Carbohydrate Polymers, 86(1): 320–327.
[34] Wang, Y., Mao, F. and Wei, X., 2012. Characterization and antioxidant activities of polysaccharides from leaves, flowers and seeds of green tea. Carbohydrate Polymers, 88(1): 146–153.

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مقاله علمی_پژوهشی

بررسی ساختار و فعالیت آنتی‌اکسیدانی کربوهیدرات خالص‌شده از ریشه گیاه *Amygdalus elaeagnifolia* Spach

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چکیده

کربوهیدرات‌ها به دلیل کاربرد در صنایع غذایی، دارویی و آرایشی دارای اهمیت هستند. ارتباط مستقیمی بین فیتوشیمی کربوهیدرات‌ها و چگونگی عملکرد و کاربرد آن‌ها در این صنایع وجود دارد. در ایران، تحقیقات کافی در زمینه بررسی کربوهیدرات‌های موجود در گونه‌های بومی و اندمیک کشور انجام نشده است. بدین منظور هدف از این پژوهش بررسی ساختار و خواص آنتی اکسیدانی کربوهیدرات گونه بومی *Amygdalus elaeagnifolia* Spach می‌باشد. ریشه این گیاه از استان فارس جمع آوری شد. در ابتدا ریشه‌ها تمیز و پیش استخراج با حلال اتانول صورت گرفت. استخراج اصلی با استفاده از آب داغ انجام گردید. جهت خالص‌سازی کربوهیدرات‌ها، مراحل مختلفی از جمله رسوب‌دهی کربوهیدرات با اتانول، حذف پروتئین‌ها با روش سواگ، به کارگیری جاذب تبادل یونی DEAE-Cellulose و در نهایت سفادکس G-100 استفاده شد. کربوهیدرات خالص شده AEC-1 نامگذاری گردید. گروه‌های عاملی موجود در AEC-1 با استفاده از طیف‌سنج فروسرخ (FT-IR) مطالعه شدند. مطالعات کمی و شناسایی نوع مونومرها به ترتیب با استفاده از کروماتوگرافی گازی متصل به آشکارساز یونیزاسیون شعله (GC-FID) و طیف‌سنج جرمی (GC-MS) انجام شد. همچنین فعالیت آنتی اکسیدانی AEC-1 با استفاده از سه نوع رادیکال آزاد، ۲،۲-دی‌فنیل-۱-پیکریل هیدرازین (DPPH)، ۲،۲′-آزینو-بیس (۳-اتیل بنزوتیازولین-٦-اسیدسولفونیک) (ABTS) و رادیکال هیدروکسیل سنجیده شد. درصد خلوص AEC-1 ۰/٦۷ ± ۹٤/۰۸ بدست آمد. بررسی گروه‌های عاملی با استفاده از FT-IR نمایانگر آن است که این کربوهیدرات دارای گروه‌های اسیدی، کربن‌های آنومری آلفا و مونوساکاریدهای پیرانوزی می‌باشد. بررسی کروماتوگرام‌های GC-FID و GC-MS نشان داد که AEC-1 دارای مونوساکاریدهای آرابینوز، گالاکتوز، مانوز، گلوکز، فروکتوز و گلوکرونیک اسید به ترتیب با درصدهای ۳۱/۵۲٪، ۲٦/۱۰٪، ۱۹/۰۰٪، ۱٦/۱۷٪، ٤/٦۳٪ و ۲/۵٦٪ است. بررسی‌های حاصل از عملکرد آنتی اکسیدانی این کربوهیدرات در مقابل رادیکال‌های DPPH، ABTS و OH نشان می‌دهد که با افزایش میزان غلظت AEC-1 فعالیت مهار کنندگی این کربوهیدرات افزایش می‌یابد. بر اساس نتایج، کربوهیدرات خالص شده یک هتروپلی‌ساکارید اسیدی با فعالیت آنتی اکسیدانی نسبتا قابل قبول است.