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Extraction and purification of proteins from Prunus armeniaca seeds

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ABSTRACT

Prunus armeniaca (apricot) is a nutrient-dense plant widely cultivated for its edible fruit and valuable seed components. In addition to supplying essential vitamins, minerals, and antioxidants, apricot seeds are a rich yet underexplored source of plant proteins with potential applications in food, cosmetics, and pharmaceutical industries. This study aimed to extract, purify, and characterize proteins from P. armeniaca seeds, evaluate their biological activity, and compare protein content between seeds collected from warm and cold climatic regions. Seed proteins were extracted using four different buffer systems, and purification was achieved by ammonium sulfate precipitation. The degree of purity was assessed using SDS-PAGE, while protein concentration was quantified spectrophotometrically. Functional properties were examined through antibacterial assays and methylene blue adsorption tests. Higher protein concentration was observed in the seeds of high-temperature regions compared to lower-temperature seeds. For high-temperature seed, the highest protein concentration (13.1 mg/µl) was achieved in Tris-HCl buffer, while distilled water gave the highest concentration (9.098 mg/ µl) in lower temperature seed. Optimal protein recovery was obtained at 65 % and 70 % ammonium sulfate saturation. However, the purified proteins showed no significant antibacterial activity and failed to remove methylene blue, suggesting limited antimicrobial and adsorptive potential. Comparative assessment revealed higher protein content in seeds from warmer regions, indicating temperature-related variation in protein synthesis. This work highlights P. armeniaca seeds as an important yet underutilized source of plant proteins. Environmental factors strongly influence protein yield, and further structural and functional studies are warranted to explore potential biotechnological and therapeutic applications.

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INTRODUCTION

The genus *Prunus* comprises a large and diverse group of fruit-bearing plants belonging to the family *Rosaceae*, which includes several economically important species, such as the peach (P. persica), plum (P. domestica), cherry (P. avium), and apricot (P. armeniaca) [1, 2]. *Prunus armeniaca*, commonly known as apricot, is a small deciduous tree that typically grows 8-12 m in height with a trunk diameter of up to 40 cm and a dense, spreading canopy. It is believed that *P. armeniaca* originated in Central Asia and China, and was later cultivated in Armenia, the Middle East, and the Mediterranean Basin [3, 4]. Botanically, apricot trees bear ovate leaves (5-9 cm long) with serrated margins and produce white to pinkish flowers that bloom in early spring before leaf emergence. The characteristic fruit is a yellow-to-orange drupe, resembling a small peach, with smooth or slightly pubescent skin, a firm flesh, and a single seed enclosed within a hard endocarp [5, 6].

P. armeniaca thrives best in temperate and Mediterranean climates with cold winters and dry summers. The species requires a chilling period of 300-900 chilling units for proper dormancy and flowering. Despite being relatively tolerant to low temperatures (up to –30 °C), early spring frosts pose a major threat to its flowers, often reducing fruit yield [7, 8]. Globally, apricot cultivation covers significant agricultural areas in Turkey, Iran, Italy, Algeria, and Uzbekistan, with the United States (especially California) being a leading producer outside the Mediterranean region. The world's apricot production is approximately 3.4 million metric tons [9, 10]. Apricots are nutritious, rich in carbohydrates (11-13 %), water (≈85 %), and essential micronutrients, including calcium, phosphorus, iron, and potassium. They are a rich source of antioxidants such as carotenoids (vitamin A) and ascorbic acid (vitamin C). The fruits are commonly consumed fresh, dried, or processed into jams, juices, and confectionery products. Dried apricots, mainly produced in Turkey, are valued for their concentrated nutrient profile and are an excellent source of vitamin E, potassium, and dietary fibre [11, 12].

Medicinally, *P. armeniaca* has been recognized for centuries in traditional systems for its therapeutic potential. Various plant parts, such as the bark, flowers, fruit, and particularly the seeds (kernels), exhibit diverse pharmacological properties such as anti-inflammatory, antiseptic, demulcent, expectorant, and antioxidant activities [13, 14]. The seeds of *P. armeniaca* contain bioactive compounds, like amygdalin (a cyanogenic glycoside), proteins, essential amino acids, and a high proportion of unsaturated fatty acids, mainly oleic and linoleic acids, which make them valuable for both nutritional and pharmaceutical applications [14]. Seed oil extracted from apricot kernels is widely used in the cosmetic industry for skin protection, and the kernel meal is a rich source of proteins (14-45%) with a balanced amino acid composition [10, 15].

It has been reported that apricot extract has promising antimicrobial and antioxidant properties. Both bitter and sweet kernels demonstrate inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Candida* species. Methanolic and aqueous extracts of sweet kernels possess significant phenolic content and antioxidant activity [10, 15]. Moreover, the presence of amygdalin and its derivative laetrile (purported "vitamin B17") has been associated with anticancer potential through mechanisms linked to apoptosis induction in tumor cells [16-18]. *Prunus armeniaca* exhibits anticancer potential through bioactive compounds that induce apoptosis and inhibit tumor cell proliferation. Furthermore, *P. armeniaca* shows potential protective effects against various cancers due to its rich content of antioxidants, phenolic compounds, and vitamins that help reduce oxidative stress and UV-induced DNA damage [19-

21]. Although clinical validation remains limited, such findings highlight the importance of apricot seeds as a natural therapeutic agent.

Despite extensive research on the phytochemical constituents of *P. armeniaca*, few studies have focused on the protein content and bioactivity of kernel-derived proteins, especially their potential antibacterial effects. Given the global emphasis on discovering plant-based antimicrobial agents to combat antibiotic resistance, proteins and peptides isolated from fruit seeds may represent promising candidates. Furthermore, the extraction buffer significantly influences the yield and functionality of recovered proteins, yet comparative data for *P. armeniaca* kernels remain scarce. Therefore, the present study was designed to (i) extract and quantify proteins from *P. armeniaca* seeds using four different extraction solutions, (ii) compare the protein yield between two seed varieties, and (iii) evaluate the antibacterial activity of the purified protein fractions.

2. MATERIALS AND METHODS

2.1 Extraction of proteins

Four different extraction buffers were employed to isolate proteins from *Prunus armeniaca seeds*: Tris-HCl (0.01 M, pH 8.0), sodium acetate (0.01 M, pH 4.0), calcium chloride (0.1 M), and distilled water (control extraction medium). All prepared buffers and solutions were stored at 4 °C.

Two different types of apricot seeds were used: the seeds (large) collected from high-temperature regions such as Punjab, and the seeds (small) obtained from colder regions, such as Gilgit-Baltistan and Swat. These different seed types allowed evaluation of temperature-related differences in protein content between the two seed types.

2.2 Procedure

After removing the seed coat, the seed kernels were finely ground into powder using a clean spatula. The kernel powder was then suspended in 2ml of each extraction buffer (Tris-HCl, sodium acetate, and distilled water) and allowed to stand for 30 minutes. For protein solubilization, the suspensions were magnetically stirred at 40 rpm for 20 minutes. The mixtures were centrifuged at 15,000 RPM for 15 minutes. The resulting supernatants, containing the extracted proteins, were collected in sterile Falcon tubes and stored at 4°C for further analysis.

2.3 Precipitation of protein

The protein pool was created by mixing an equal volume of supernatant from all four extraction mixtures. This pooled sample was used to purify the extracted proteins through ammonium sulfate precipitation. A 5 mL aliquot of the pooled extract was dispensed into each of several sterile Falcon tubes to prepare different ammonium sulfate saturation levels. The required quantities of ammonium sulfate were carefully weighed and added to each tube to achieve saturations of 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%, corresponding to 0.53 g, 0.27 g, 0.28 g, 0.29 g, 0.30 g, 0.31 g, 0.35 g, and 0.39 g of ammonium sulfate, respectively. The mixtures were thoroughly mixed until complete dissolution of the salt to ensure uniform precipitation before proceeding with centrifugation and further purification steps. The solution was then magnetically stirred at 400 RPM without heat and was left for 30 minutes for precipitation of proteins, followed by centrifugation at 14,000 RPM for 15 minutes at 4°C. The supernatant was removed, and the pellet was re-suspended in 50 µl of Tris-HCl buffer, pH 8.0 and stored at 4°C. The process was repeated for all, 20%, 30%, 40%, 50%, 60% and 70% saturations.

2.4 Quantification of protein

A spectrophotometer was used to check protein concentration. The lid factor 10 was used. The lid factor 10 is used to quantify the samples. Tris-HCl buffer is used as a blank, as the pellets were suspended in the Tris-HCl buffer.

2.4.1 SDS-PAGE

2.4.1.1 Reagent preparation

1X electrode buffer was prepared using the reagents: 3.9 g of Tris-base (25 mM), 14.4 g of glycine (192 mM), and 1 g of SDS (0.1%) in one liter of distilled water. The solution was mixed thoroughly and stored at room temperature for use in electrophoresis experiments.

The non-reducing sample buffer was prepared using the components: 1.275 mL of 0.06 M Tris-HCl, 2 mL of 10% SDS, 1 mL of 10% glycerol, 0.25 mL of 0.025% bromophenol blue, and 4.8 mL of distilled water and obtained a final volume of 10 mL. β-mercaptoethanol was intentionally excluded from the mixture to maintain non-reducing conditions. The solution was mixed thoroughly and stored at 4 °C until use. For reducing buffer, 50ul of B-mercaptoethanol was added to 950 µl of non-reducing buffer.

For gel staining, a staining solution was prepared with a final volume of 100 mL with the composition: 10% (v/v) acetic acid, 50% (v/v) methanol, and 0.4 g of Coomassie Brilliant Blue dye. For the de-staining, a 50% methanol solution was used.

A 15% separating gel with a final volume of 11 mL was prepared for SDS–PAGE analysis. The gel solution was composed of 2.47 mL of distilled water, 5.5 mL of 30% acrylamide-bisacrylamide solution, 2.75 mL of 1.5 M Tris buffer (pH 8.8), 110 µL of 10% SDS, 110 µL of 10% ammonium persulfate (APS), and 11 µL of TEMED.

The stacking gel was prepared at a concentration of 4% with a final volume of 5 mL. The composition of the stacking gel solution was as follows: 3.0 mL of distilled water, 0.67 mL of 30% acrylamide/bis-acrylamide solution, 1.25 mL of 0.5 M Tris-HCl buffer (pH 6.8), 50 µL of 10% SDS, 50 µL of 10% ammonium persulfate (APS), and 5 µL of TEMED.

2.4.1.2 Procedure

Two SDS-PAGE gels were prepared and run separately: one for proteins extracted using the four different extraction buffers and another for protein pellets obtained from various ammonium sulfate saturation levels. For sample preparation, 30 µL of each protein extract was mixed with 5 µL of sample loading buffer in Eppendorf tubes and subjected to heat denaturation at 99 °C for 4 minutes. The electrophoresis tank was filled with 1×electrode buffer, and samples were carefully loaded into the wells. The gel was initially run at 100 V for 30 minutes to allow proper stacking, followed by 150 V for 120 minutes to achieve efficient protein separation. After electrophoresis, the gel was immersed in Coomassie Brilliant Blue staining solution for 25 minutes and transferred to a destaining solution and left overnight until a clear background and distinct bands were obtained. The molecular weights of the separated proteins were determined by measuring their migration distances relative to standard protein markers and plotting the logarithm of

molecular weight (log MW) against migration distance to estimate the molecular weights of the purified proteins.

2.7 Methylene blue removal test

The powder of *Prunus armeniaca* of varying amounts (0.01, 0.03, 0.06, 0.1, and 0.12 g) was added to 5 mL of a 1% methylene blue stock solution. The mixtures were incubated for 40 minutes and then centrifuged at 1,000 RPM for 10 minutes. The supernatants were collected, and their absorbance was measured using a spectrophotometer at the wavelength range of 500-700 nm.

2.8 Antibacterial Assay

Luria-Bertani (LB) agar was used to assess the antibacterial activity of purified seed proteins extracted from *Prunus armeniaca* (apricot) against *Enterococcus lactis* R13 and *Enterococcus durans* S2c. The bacterial strains were first cultured in 5 mL of LB broth within sterile Falcon tubes under aseptic conditions in a laminar flow hood and incubated at 37 °C for 24 hours to obtain an active bacterial inoculum, followed by lawn preparation on LB agar plates. The antibacterial potential of the purified protein was evaluated using the agar well diffusion method. For this purpose, 5.25 g of LB agar was dissolved in 150 mL of distilled water and sterilized by autoclaving at 121 °C for 15 minutes. The medium was cooled to approximately 45-50 °C, poured evenly into six sterile Petri plates, and allowed to solidify for 25 minutes. The surface of each plate was inoculated with bacterial culture using sterile cotton swabs to ensure uniform lawn formation. Wells were created using a sterile agar borer, and 25 µL of purified protein was added to each well. Plates were incubated at 37 °C for 12-24 hours, and zones of inhibition (antibacterial activity) were measured in millimeters to determine protein efficacy.

3. RESULTS AND DISCUSSION

3.1 Extraction of proteins

Two distinct types of *Prunus armeniaca* seeds from the same plant species were selected based on their geographical temperature zones. SDS-PAGE analysis of protein extracted from high-temperature areas showed multiple well-defined bands, indicating higher protein yield and diversity (**Figure 1A**), while the protein profile of seeds from low-temperature areas exhibited fewer and lighter bands, suggesting lower protein concentration (**Figure 1B**).

The electrophoretic pattern revealed that the protein concentration in seeds collected from high-temperature regions was significantly higher than that in seeds from low-temperature areas, indicating that environmental temperature may influence protein accumulation in *P. armeniaca* seeds. Furthermore, in seed 1, Tris-HCl buffer (pH 8.0) yielded the highest protein concentration (13.1 mg/**µl**) from *Prunus armeniaca* seeds, followed by an equal concentration in both calcium chloride and sodium acetate (**10.4** mg/**µl**), while distilled water gave the lowest yield (**9.579** mg/**µl**) **(Figure 2A)**.

Moreover, the results of seed 2 protein extraction showed varied concentrations of protein, with the highest concentration found in distilled water (9.098 mg/µL), followed by Tris-HCl buffer (7.876 mg/µL), calcium chloride (5.752 mg/µL), and sodium acetate (4.880 mg/µL) **(Figure 2B)**.

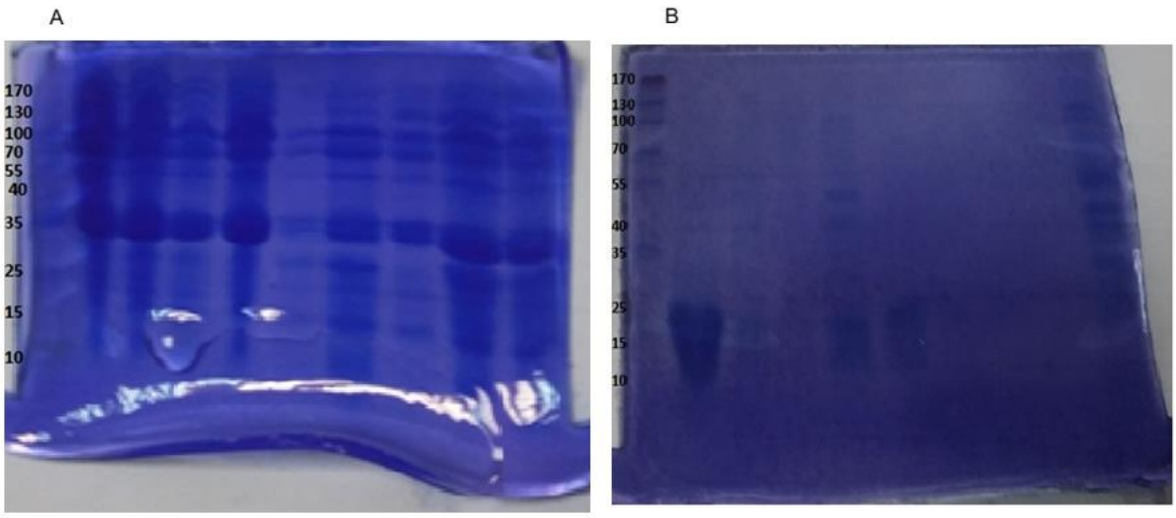

Figure 1: SDS-PAGE analysis of protein extracts from *Prunus armeniaca* (apricot) seeds. (**A**) Protein profile of seeds from high-temperature areas (**B**) Protein profile of seeds from low-temperature areas. Molecular weight markers (kDa) are indicated on the left side of each gel.

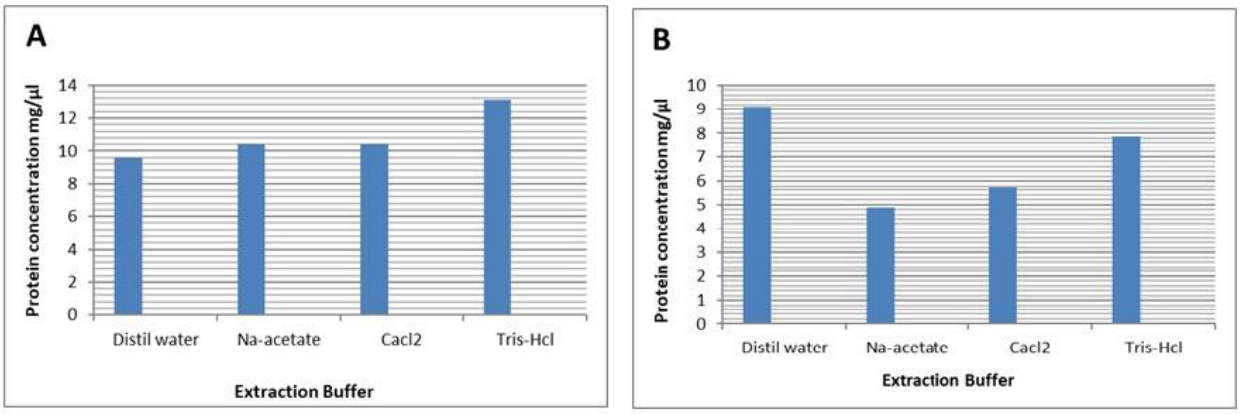

Figure 2: Quantification of protein concentration in *Prunus armeniaca* seed extracts obtained using different extraction buffers. **(A)** Protein profile of seed 1 from high-temperature areas and **(B)** Protein profile of seed 2 from low-temperature areas.

3.2 Precipitation of protein

After the extraction and comparison of crude proteins between the two types of seeds with respect to their protein level, we continued our work with seed 2, belonging to the low-temperature area. We continued with the Tris-HCl extraction buffer because it gave the highest protein level in the purification step. Seed powder was mixed in Tris-HCl and then proceeded with different saturation concentrations, i.e., 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80% and 90%. After centrifugation, the pellets were analyzed using SDS-PAGE to check the presence of the purified protein of interest. A band of 25 kDa size was observed on the gel (**Figure 3**).

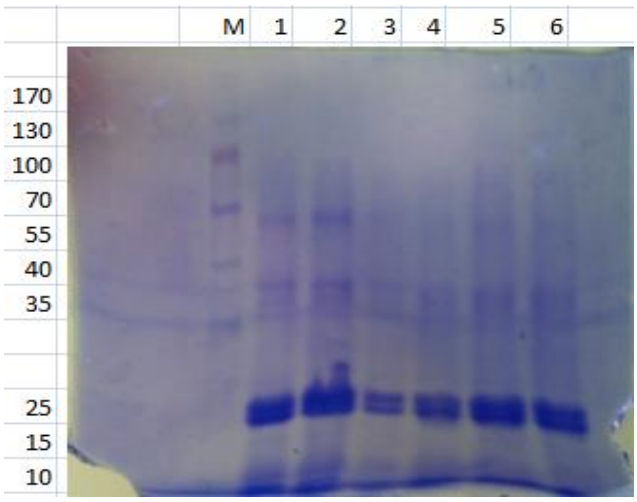

Figure 3: Gel of Pure protein of seed 2

3.4. Molecular weight of Gel

After getting pure protein by analyzing SDS-PAGE gel, we estimated the molecular weight (M.W) of the purified protein, based on its migration relative to the standard marker set (**Figure 4**).

The calibration curve showed a strong linear relationship between migration distance and log MW. Using the regression equation, the band corresponding to the purified fraction was calculated to have a molecular weight of approximately 13.87 kDa. This value suggests that the isolated protein is a small, well-resolved component of the seed extract, consistent with the pattern observed in the surrounding lanes.

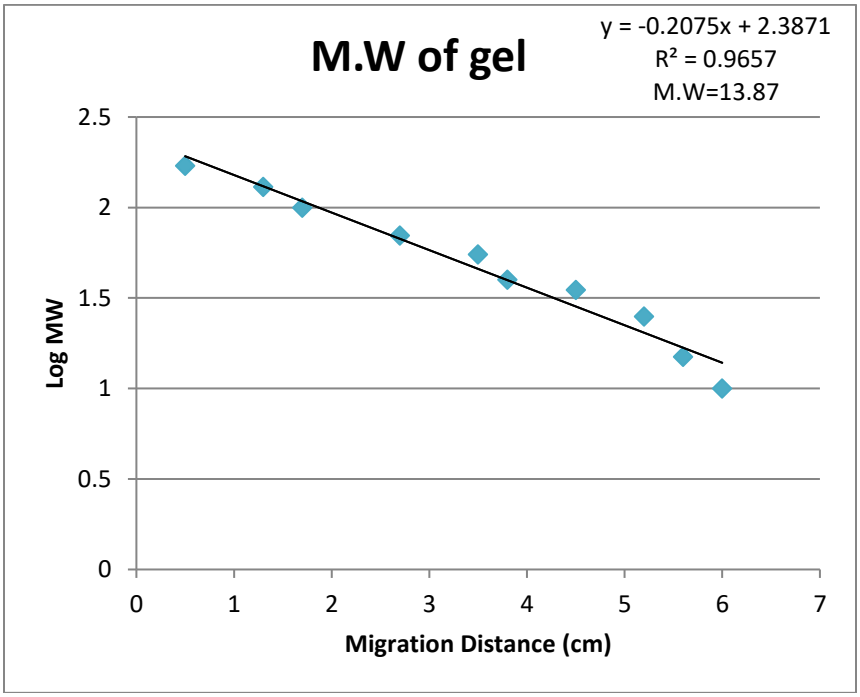

Figure 4: Molecular weight of Gel

3.5 Removal of Methylene Blue

The assessment of dye-removal ability showed that *P. armeniaca* seeds were unable to remove methylene blue under the tested conditions. The seed extract did not show any visible reduction in dye intensity.

3.6 Antibacterial Activity

To check the antibacterial activity of the protein of interest, the agar well diffusion assay was performed. Two strains, R13 and S2c bacterial cultures, were treated with the protein. No zone of inhibition was observed in any of the plates (**Figure 5**). The results showed that the protein extract did not show any inhibitory or antimicrobial activity against the tested bacterial strains. These findings are consistent with previous reports indicating that proteins from *P. armeniaca* do not exhibit measurable antibacterial effects [22, 23].

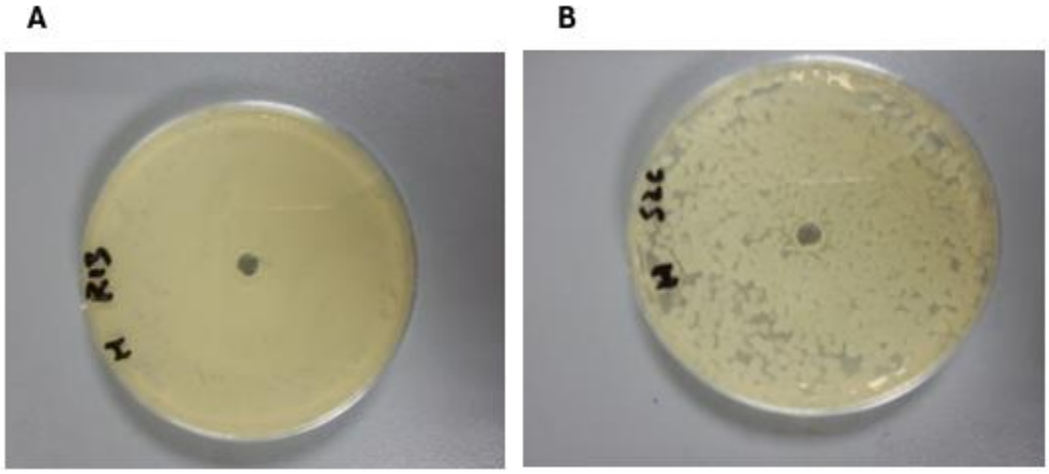

Figure 5: Antibacterial activity of purified *Prunus armeniaca* seed protein against (**A**) *Enterococcus lactis* (R13) and (**B**) *Enterococcus durans* (S2c)

A large amount and concentration of protein were extracted from the seeds of *P.armeniaca* in this study, and the findings are inconsistent with the past studies that reported the high protein content (34.3%) in the apricot kernel press cake left after the extraction of oil. They reported that ammonium sulfate precipitation between 30 and 70 per cent saturation recovered 87.34 per cent of the extracted enzyme and increased the specific activity and purification fold to 29.07 U/mg protein and 2.92, respectively. Their findings demonstrate the efficiency of this saturation range for enriching the target protein [24, 25]. In the present study, the protein of interest also precipitated within this window, specifically at 65 to 70 per cent saturation, confirming that ammonium sulfate is suitable for obtaining a purified fraction from *P. armeniaca* seeds.

4. CONCLUSION

The present study highlights the significant nutritional potential of *Prunus armeniaca* seeds. The proteins extracted from apricot kernels exhibit promising bioactive properties that may be harnessed for medicinal purposes when utilized appropriately. In addition to their nutritional richness, *P. armeniaca* fruits provide essential vitamins and minerals that support overall health and well-being. Further research focusing on the purification, structural characterization, and mechanistic evaluation of *P. armeniaca* proteins is recommended to fully elucidate their biomedical potential and safety profile for future clinical and commercial use.

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Authors Contribution
Haseeb Ahmed – Literature Review, Primer design, Experiment Design, Experimental Work, Result Analyses
Ammar Azmat – Primer design, Experiment Design, Experimental Work, Result Analyses

Conflict of interest statement
The author declares that they have no conflict of interest.

REFERENCES

1. Gharaghani, A. and S. Solhjoo, *Varietal diversification of stone fruits*, in *Production technology of stone fruits*. 2021, Springer. p. 1-56.
2. Liu, Z., et al., *Developmental mechanisms of fleshy fruit diversity in Rosaceae.* Annual Review of Plant Biology, 2020. **71**(1): p. 547-573.
3. Seethapathy, P., et al., *Diseases, pests, and disorders in plum: diagnosis and management*, in *Handbook of plum fruit*. 2022, CRC Press. p. 133-176.
4. Hussain, S.Z., et al., *Fruits grown in highland regions of the Himalayas*. 2021: Springer.
5. Mahendiran, G., S. Lal, and O. Sharma, *Pests and Their Management on Temperate Fruits: (Apple, Pear, Peach, Apricot, Cherry, Persimmon, Walnut, Olive, Kiwifruit and Strawberry).* Trends in Horticultural Entomology, 2022: p. 891-941.
6. Hussain, S.Z., et al., *Apricots (Prunus armeniaca)—Morphology, taxonomy, composition and health benefits*, in *Fruits Grown in Highland Regions of the Himalayas: Nutritional and Health Benefits*. 2021, Springer. p. 91-102.
7. Bartolini, S., R. Massai, and R. Viti, *The influence of autumn-winter temperatures on endodormancy release and blooming performance of apricot (Prunus armeniaca L.) in central Italy based on long-term observations.* The Journal of Horticultural Science and Biotechnology, 2020. **95**(6): p. 794-803.
8. Djaman, K., et al., *Chilling and heat accumulation of fruit and nut trees and flower bud vulnerability to early spring low temperatures in New Mexico: meteorological approach.* Sustainability, 2021. **13**(5): p. 2524.
9. Uzundumlu, A.S., T. Karabacak, and A. Ali, *Apricot production forecast of the leading countries in the period of 2018-2025.* Emirates Journal of Food & Agriculture (EJFA), 2021. **33**(8).
10. Al-Soufi, M.H., et al., *A review with updated perspectives on nutritional and therapeutic benefits of apricot and the industrial application of its underutilized parts.* Molecules, 2022. **27**(15): p. 5016.
11. Karatas, N., *Evaluation of nutritional content in wild apricot fruits for sustainable apricot production.* Sustainability, 2022. **14**(3): p. 1063.
12. Alajil, O., et al., *Nutritional and phytochemical traits of apricots (Prunus armeniaca L.) for application in nutraceutical and health industry.* Foods, 2021. **10**(6): p. 1344.
13. Koriem, K.M.M., *Phytochemical screening, chemical constituents, traditional medicine usage, pharmacological effect, metabolism and pharmacokinetics of Semen armeniacae.* Biointerface Research in Applied Chemistry, 2022. **12**(3): p. 3186-3197.
14. Kitic, D., et al., *Anticancer potential and other pharmacological properties of Prunus armeniaca L.: an updated overview.* Plants, 2022. **11**(14): p. 1885.

15. Akhone, M.A., et al., *Apricot kernel: bioactivity, characterization, applications, and health attributes.* Foods, 2022. **11**(15): p. 2184.
16. Mahmoudi, E., et al., *Prunus armeniaca effects on expression of genes related to apoptosis in human breast cancer cells.* Translational Medicine Communications, 2019. **4**(1): p. 5.
17. Bora, A., et al., *Amygdalin laetrile-a nascent vitamin B17: A review.* Int. J. Res. Med. Sci, 2021. **9**: p. 2160-2166.
18. Barakat, H., et al., *Amygdalin: a review on its characteristics, antioxidant potential, gastrointestinal microbiota intervention, anticancer therapeutic and mechanisms, toxicity, and encapsulation.* Biomolecules, 2022. **12**(10): p. 1514.
19. Yousaf, A., et al., *Gastric cancer associated risk factors and prevalence in Pakistan.* ASEAN Journal of Science and Engineering, 2021. **1**(2): p. 73-78.
20. Hosny, S., et al., *Prunus Armeniaca L. seed extract and its amygdalin containing fraction induced mitochondrial-mediated apoptosis and autophagy in liver carcinogenesis.* Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents), 2021. **21**(5): p. 621-629.
21. Bailly, C., *Anticancer properties of Prunus mume extracts (Chinese plum, Japanese apricot).* Journal of ethnopharmacology, 2020. **246**: p. 112215.
22. Ginovyan, M., M. Petrosyan, and A. Trchounian, *Antimicrobial activity of some plant materials used in Armenian traditional medicine.* BMC complementary and alternative medicine, 2017. **17**(1): p. 50.
23. Erdogan-Orhan, I. and M. Kartal, *Insights into research on phytochemistry and biological activities of Prunus armeniaca L.(apricot).* Food research international, 2011. **44**(5): p. 1238-1243.
24. Abd El-Aal, M., M. Hamza, and E. Rahma, *In vitro digestibility, physico-chemical and functional properties of apricot kernel proteins.* Food chemistry, 1986. **19**(3): p. 197-211.
25. Kiralan, M., et al., *Apricot (Prunus armeniac a L.) Oil*, in *Fruit oils: Chemistry and functionality*. 2019, Springer. p. 505-519.